Carbamate, Thiocarbamate and Substituted Urea Compounds

CARCINOGENICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS. OTHER BIOLOGICAL PROPERTIES. ACTIVATING METABOLISM. ENVIRONMENTAL SIGNIFICANCE.

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5 2 1 6 1 Historical Background Carbamates are esters or salts of a simple organic chemical, carbamic acid, NH_2COOH Substitution of one or both oxygen atoms of carbamate with sulfur gives rise to thiocarbamate or dithiocarbamate, respectively. Replacement of the alkoxy group of carbamate by an amino group yields urea type compounds. Carbamate and related compounds have long been known to possess a variety of toxicological and pharmacological properties A naturally occurring carbamate alkaloid present in the seeds of Calabar bean was reportedly used several hundreds of years ago by the natives (the Efiks) of Old Calabar along the coastline of West Africa in their witchcraft trials The accused was reportedly forced to swallow a milky potion containing macerated Calabar bean seeds and ordered to walk around until justice was served — he either regurgitated the potion, recovered and was declared innocent or he died quickly of the toxic effects thus establishing his guilt (1) ~~The discovery by Europeans of~~ The native use of the bean seeds initiated a flurry of research in Europe which eventually led to the identification and synthesis of the active carbamate alkaloid, ~~which was named~~ physotigmine. Extensive investigations of physotigmine, through its neurotoxic effects, led to the elucidation of the mechanism of transmission of nerve impulse and laid the foundation of toxicology and pharmacology of carbamate compounds Studies of physotigmine analogs stimulated an intensive interest in the search and development of carbamate and related compounds as pesticides. The historical development of carbamate, thiocarbamate and urea type pesticides has been

described (2-4). To date, hundreds of these compounds have been developed, many of which are used commercially in very large amounts

Urethan (ethyl carbamate), one of the simplest carbamates, was first synthesized from urea and ethanol by Wöhler in 1840. It has since found uses in human and veterinary medicine, (as) industrial chemical intermediate, solubilizer and co-solvent. Like many carbamates, urethan has narcotic action It was formerly used as a hypnotic in humans. In fact, it was recommended as "a very safe hypnotic, . excellent for children . " in a pharmacology textbook published in 1940 (5). At high doses (in the order of 1 g/kg), urethan was used as an anesthetic for laboratory animals and it was at these high doses that the carcinogenic effect of urethan was discovered in 1943 In a study designed to investigate the effect of X-ray irradiation on the initiation of skin tumors in C3H mice, Nettleship, Henshaw and Meyer (6) noted an unexpectedly high incidence of multiple lung tumors in both experimental and control animals. On further examination, it occurred to them that during irradiation the mice had been under urethan anesthesia, and it was urethan treatment that was responsible for the lung tumorigenesis. For some time, urethan was considered as a specific lung carcinogen However, in 1953, it was independently demonstrated by Graffi et al (7) and Salaman and Roe (8) that urethan, when applied to mouse skin, ~~exhibited peculiar properties. It~~ was incapable of inducing skin tumors by itself but could so modify the skin that subsequent applications of croton oil (a promotor) resulted in the appearance of papillomas. Interestingly, the skin-tumor-initiating activity could also be demonstrated by oral

administration of urethan (9). These findings helped firmly substantiate the concept of initiation and promotion in the process of two-stage skin carcinogenesis, ~~etc.~~ Intrigued by reports of skin carcinogenicity of urethan, Tannenbaum and associates (10-14) undertook a series of long-term studies in several strains of mice and demonstrated that urethan has a much broader spectrum of carcinogenic action In addition to lung adenomas, it induced or potentiated mammary carcinomas, malignant mesenchymal tumors in the interscapular fat pad, and cystadenomas of the harderian gland These results have been confirmed by various investigators (see Section 5 2 1 6 3) using different species and strains Furthermore, several other tissues of some strains have also been found susceptible to the carcinogenic action of urethan thus clearly establishing urethan as a multipotential agent . Urethan is now one of the most extensively used experimental carcinogens.

The study of the structure-activity relationships of urethan and related compounds was initiated by the pioneering studies of Larsen (15, 16) and Berenblum et al (17) Small changes in the molecular structure may have profound effect on the carcinogenic potential of the carbamates To date, close to a hundred carbamates and related compounds have been tested for carcinogenicity. Of great importance is the necessity to assess the carcinogenic risk of carbamates and related compounds used as pesticides. The ever-increasing demand for pest control, the dwindling supply of naturally occurring pesticides and the recent governmental banning of certain organochlorine pesticides have placed an increasingly prominent role on carbamates and related compounds

in crop and animal protection. A large-scale preliminary carcinogenicity study on 130 commercial pesticides was undertaken by the National Cancer Institute at Bionetics Laboratories in 1963 and completed in 1968 Close to 30 carbamate, thiocarbamate and substituted urea compounds were included in the study Of these compounds, three (Diallate, Ethyl selenac, and Potassium bis-2-hydroxyethyl-dithiocarbamate) were considered carcinogenic Six (Sodium diethyldithiocarbamate, Monuron, Ethyl tellurac, Ledate, Disulfiram, Zectran) had marginal activity and required further evaluation (18, 19) (see Section 5 2.1 6 3). Further studies of some of these and related compounds have been completed or are in progress in the current carcinogenesis testing program of the National Toxicology Program This chapter reviews these and other carcinogenicity studies with special emphasis on the structure-activity relationships

5 2 1 6 2 Physical and Chemical Properties and Biological Effects

5.2 1 6 2.1 Physical and Chemical Properties The physical and chemical properties of carbamate, thiocarbamate and substituted urea compounds have been described in detail in several reviews (20-23) and monographs (2, 3, 24). The physical constants of some of the more well known compounds are summarized in Table CXIII. In general, simple alkyl carbamates are water soluble and slightly volatile, both the solubility and volatility decrease with the increase of the size of the alkyl group and/or replacement of the amino hydrogens by alkyl or aryl groups

The chemical properties of simple alkyl carbamates (20) and carbamate pesticides (3, 24) have been reviewed The basic backbone of all these compounds

Table CXIII

Physical Constants of Carbamates, Thiocarbamates and Substituted Urea Compounds[a]

Compound[b]	m. p.	b. p.	Density	Refractive index	Vapor pressure	Solubility
Urethan [Ethyl carbamate]	48-50°[a]	182-184°	—	—	volatile	Soluble in water (1 g in 0.5 ml at 25°), ethanol, chloroform, ether
Methyl carbamate	54°	177°	—	$n_D^{56} = 1.4125$	—	Soluble in water, ethanol, ether
n-Propyl carbamate	60°	196°	—	—	1 mm Hg (52.4°)	Soluble in water, ethanol, ether
Zectran [Mexacarbate, 4-(dimethylamino)-3, 5-dimethylphenyl N-methylcarbamate]	85°	—	—	—	0.1 mm Hg (139°)	Practically insoluble in water, (100 ppm at 25°), soluble in acetone ethanol, benzene, acetonitrile, methylene chloride
Propoxur [Baygon, 2-isopropoxyphenyl N-methylcarbamate]	84-87°	—	—	—	0.01 mm Hg (120°)	Slightly soluble in water (0.2% at 20°), soluble in most organic solvents
Carbaryl [Sevin, 1-naphthyl N-methylcarbamate]	142°	—	$d_{20}^{20} = 1.232$	—	4.1×10^{-5} mm Hg (25°)	Practically insoluble in water (40 ppm at 25°), moderately soluble in dimethylformamide, acetone, isophorone

Table CXIII, continued

Carbofuran [Furadan, 2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl N-methylcarbamate]	153-154°	–	d_{25}^{20} = 1.180	–	2×10^{-5} mm Hg (33°)	Sparingly soluble in water (0.07%) at 25°); soluble in dimethylformamide, dimethyl sulfoxide, N-methyl-2-pyrrolidone
Propham [IPC, Isopropyl N-phenylcarbamate]	87-87.6°	–	–	n_D^{91} = 1.4989	–	Practically insoluble in water (32-250 ppm at 20-25°), soluble in most organic solvents
Chloropropham [CIPC, Isopropyl N-(3-chlorophenyl)-carbamate]	40.7-41°	–	–	n_D^{20} = 1.5395	10^{-6}-10^{-5} mm Hg (25°)	Practically insoluble in water (88 ppm at 25°), miscible with most organic solvents
Primicarb [2-(dimethylamino)-5, 6-dimethylpyrimidin-4-yl dimethylcarbamate]	90.5°	–	–	–	3×10^{-5} mm Hg (30°)	Slightly soluble in water (0.27% at 25°), soluble in most organic solvents
Aldicarb [Temik, 2-methyl-2-(methylthio)-proprionaldehyde O-(methylcarbamoyl)-oxime]	98-100°	–	d_{20}^{25} = 1 195	–	1×10^{-4} mm Hg (25°)	Slightly soluble in water (0.9% at 30°), soluble in most organic solvents
Diallate [Avadex, S-(2, 3-dichloroallyl) N, N-diisopropylthiocarbamate]	—	150° (9 mm Hg)	$d_{15.6}^{25}$ = 1.188	–	1.5×10^{-4} mm Hg (25°)	Practically insoluble in water (14 ppm at 25°), miscible with most organic solvents
Sulfallate [CDEC, 2-chloroallyl N, N-diethyldithiocarbamate]	—	128-130° (9 mm Hg)	d^{25} = 1.088	n_D^{52} = 1.5822	2.2×10^{-3} mm Hg (20°)	Practically insoluble in water (100 ppm at 25°), miscible with most organic solvents

Thiram [TMTD, tetramethylthiuram disulfide]	155-156°	–	$d^{20} = 1.29$	–	–	Practically insoluble in water (30 ppm at room temperature), soluble in acetone, chloroform
Disulfiram [Tetraethylthiuram disulfide, Antabuse]	70°	–	$d = 1.30$	–	–	Sparingly soluble in water (0.02%), soluble in ethanol, ether, acetone, benzene, chloroform, carbon disulfide
Monuron [N'-(4-chlorophenyl)-N, N-dimethylurea]	174-175°	–	$d_{20}^{20} = 1.27$	–	5×10^{-7} mm Hg (25°)	Sparingly soluble in water (0.023% at 25°), slightly soluble in polar organic solvents such as acetone (5.2% at 27°)
Diuron [N'-(3, 4-dichlorophenyl)-N, N-dimethylurea]	158-159°	–	–	–	3.1×10^{-6} mm Hg (50°)	Practically insoluble in water (42 ppm at 25°), slightly soluble in polar organic solvents such as acetone (5.3% at 27°)

[a]Data summarized from H. Martin, "Pesticide Manual", British Crop Protection Council, 1973, 3rd edn, IARC, IARC Monog. Vol. 7, International Agency for Research on Cancer, Lyon, France, 1974, IARC, IARC Monog. Vol. 12, International Agency for Research on Cancer, Lyon, France, 1976, R. J. Kuhn and H. W. Dorough, "Carbamate Insecticides Chemistry, Biochemistry and Toxicology", CRC Press, Cleveland, Ohio, 1976.

[b]The structural formulas are depicted in Tables CXXI, CXXIII and CXXVIII.

is carbamic acid which is extremely unstable and does not exist in free form, it spontaneously decomposes to carbon dioxide and ammonia In contrast to the free acid, the esters and salts of carbamic acid are quite stable. The thermal stability of carbamates increases with the extent of N-substitution Thus, N, N-disubstituted carbamates are quite resistant to thermal decomposition, N-monosubstituted carbamates decompose at elevated temperatures primarily to alkyl isocyanate, whereas unsubstituted carbamates break down to derivatives of cyanic acid without heating. In aqueous solution, carbamates are very susceptible to alkaline hydrolysis. The half-lives of Carbaryl, Zectran and Propoxur are 0 5, 2 3 and 3 1 hrs., respectively, at pH 9 3 and 25° C (3) Also, resistance to alkaline hydrolysis is substantially enhanced by alkyl N-substitution--N, N-Disubstituted carbamates are even more resistant, the hydrolytic rate of some aromatic N, N-dimethylcarbamates is as much as 10^3 to 10^7 times slower than their N-methyl counterparts. Among the phenyl N-substituted carbamates studied, resistance to alkyl hydrolysis follows the order N-phenyl < N-benzyl < N-ethyl < N-n-propyl < N-methyl Among the N, N-disubstituted compounds studied, the ranking follows the order N, N-dimethyl < N, N-diethyl < N, N-di-n-propyl < N, N-di-n-butyl < N, N-di-isopropyl In contrast to alkaline hydrolysis, carbamates hydrolyze very slowly in mildly acidic solutions Among the substituted carbamates, acetylenic carbamates are very reactive A number of diaryl acetylenic carbamates have been demonstrated to directly alkylate tissue nucleophiles, probably by substitution reactions involving loss of carbamate anion (25) The chemistry of N-hydroxy

urethan has been reviewed by Mirvish (21). In many respects, N-hydroxy urethan, N-hydroxyurea and hydroxylamine have similar chemical properties that are different from those of urethan. They all directly react with DNA under in vitro conditions, probably via the involvement of free radical reaction.

The chemical properties of dithiocarbamate and related compounds have been reviewed (2, 22-24) Dithiocarbamates are quite reactive, they chelate metals, interact with sulfhydryl groups and undergo many reactions involving the oxidation and loss of sulfur Metallic dialkyldithiocarbamates (e g , Ziram) may be oxidized under mild conditions to yield thiuram disulfide (Thiram) in a manner analogous to the formation of disulfide from mercaptans Acid hydrolysis of dialkyldithiocarbamates yields dialkylamine and carbon disulfide The chemical properties of a number of chlorinated phenyldimethylurea compounds have been described by Melnikov (24) They are stable and may withstand heating up to the melting point, without decomposition. Upon heating in the presence of alkali or mineral acids, the compounds break down to dimethylamine and chloroaniline or chlorophenyl isocyanate

5 2.1.6.2 2 Biological (other than carcinogenic) Effects Carbamate, thiocarbamate and substituted urea compounds have a wide variety of biological activities. Only the toxic, mutagenic and teratogenic effects of those compounds that have been tested or suspected for carcinogenicity are discussed in the following paragraphs.

Toxic Effects Most of the toxicity studies in this area have been devoted to the study of carbamate pesticides. The acute toxicity data of a variety of

Table CXIV

carbamates and related compounds are summarized in Table CXIV The LD_{50} values range from 0.6 mg/kg for Aldicarb to 12.0 g/kg for disulfiram The route of administration, the sex, species and the diet all have significant effects on the toxicity of these compounds. The symptoms of carbamate poisoning in mammals are essentially identical to those of organophosphorus compounds (see Section 5 2.1.4 1). The principal toxic actions are due to the inhibition of cholinesterase, thus causing excessive stimulation of the nervous system The duration of carbamate poisoning is, however, much shorter than that of organophosphorus compounds, because of the reversibility of carbamate--cholinesterase inhibition Several case histories of human overexposure to carbamate pesticides have been reported (26, 27), the exposed individuals recovered from the symptoms of carbamate poisoning in a relatively short period of time The toxicology of carbamate insecticides (3) and dithiocarbamate fungicides (22), and the peripheral neuropathic effects caused by dithiocarbamates (28) have been extensively reviewed

Mutagenic Efects. The mutagenicity of carbamates and related compounds has been extensively tested in a variety of test organisms, such as bacteria and yeast (29), molds (30, 31), higher plants (32), Drosophila (33, rev 34), cultured mammalian cells (35-38), and experimental animals (4, 39-44). The mutagenic actions of urethan and related compounds (34) and dithiocarbamate pesticides (23) have ~~recently~~ been reviewed. The current governmental banning of certain organochlorine pesticides brought about an increase in the use of carbamate pesticides and stimulated a new surge of interest in assessing

Table CXV

Mutagenicity of Carbamate, Thiocarbamate and Substituted Urea Compounds in Salmonella Strains and Correlation to Carcinogenicity

Compound	Mutagenicity[a]				Carcinogenicity[b]
	Strain TA 100 or 1535		Strain TA 98, 1537 or 1538		
	no activation	with activation	no activation	with activation	
Urethan (ethyl carbamate)	-(41, 50, 51)	-(41, 49-51) +(52)	-(41, 50, 51)	-(41, 49-52)	+
Methyl carbamate	-(50)	-(49, 50)	-(50)	-(49, 50)	-
1, 1-Diphenyl-2-propynyl-N-cyclohexylcarbamate	+(49)	n. t.	n. t.	n. t.	+
1, 1-Diphenyl-2-butynyl-N-cyclohexylcarbamate	+(49)	n. t.	n. t.	n. t.	+
1-Phenyl-1-(3, 4-xylyl)-2-propynyl N-cyclohexylcarbamate	+(49)	n. t.	n. t.	n. t.	+
Barban	-(30, 47, 100)	-(47)	-(30, 47, 100)	-(47)	n t.
Chlorbupham	-(47)	-(47)	-(47)	-(47)	n. t.
Propoxur (Baygon)	-(47, 101, 102)	-(47)	-(47, 101, 102)	-(47)	-
Carbaryl (Sevin)	-(47, 101-103)	-(47, 49, 103)	-(47, 101-103)	-(47, 49, 103)	-

Table CXV, continued

Propham (IPC)	-(47, 100)	-(47)	-(47, 100)	-(47)	-
Chloropropham (CIPC)	-(47, 100)	-(47)	-(47, 100)	-(47)	-
Benomyl (Benlate)[c]	-(55, 56) +(54)	-(55, 56)	-(54, 56)	-(56)	-
Diallate (Avadex)	-(47, 100, 104-106)	+(47, 104, 105)	-(47, 100 104, 106)	-(47, 104)	+
Sulfallate (CDEC)	-(47, 104) +(30, 106)	+(47, 104)	-(30, 47, 104, 106)	-(47, 104)	+
Triallate (Vegadex)	-(47, 104) +(56, 106)	+(47, 56, 104)	-(30, 56, 104, 106)	-(47, 56, 104)	n. t.
Sodium dimethyldithiocarbamate	+(46)	n. t.	-(46)	n. t.	n. t.
Sodium diethyldithiocarbamate	-(46)	n. t.	-(46)	n t.	-
Ziram (zinc dimethyldithiocarbamate)	-(47) +(45, 46)	-(47)	-(46, 47)	-(47)	-, ±
Ethylzimate (zinc diethyl dithiocarbamate)	-(46)	n. t.	-(46)	n. t.	-
Arsenic dimethyldithiocarbamate	+(46)	n t.	-(46)	n. t.	n. t.
Ferbam (ferric dimethyldithiocarbamate)	-(47) +(45, 46)	-(47)	-(46, 47)	-(47)	-

Ferric diethyldithiocarbamate	-(46)	n. t.	-(46)	n t.	n. t.
Thiram	+(45, 46, 48)	-(48)	-(46, 48)	+(48)	-
Disulfiram	-(46)	n. t.	-(46)	n t.	-
Nabam	-(47)	-(47)	-(47)	-(47)	-
Maneb	-(47)	-(47)	-(47)	-(47)	-, +
Zineb	-(47)	-(47)	-(47)	-(47)	-, ±
Hydroxyurea	-(41)	-(41)	-(41)	-(41)	-
Monuron	-(57, 100)	-(57) +(53)	-(57, 100)	-(57)	-, +
Diuron	-(100)	+(53)	-(100)	n t	-

[a] Mutagenicity in strain TA 100 or 1535 (missense mutants) or strain TA 98, 1537 or 1538 (frameshift mutants) either in the presence or absence of hepatic microsomal activation systems. The numbers in parentheses indicate the reference numbers. '+' = positive; '-' = negative, 'n t' = not tested.

[b] Carcinogenicity. '+' = positive, '-' = negative, '±' = equivocal, 'n. t' = not tested. See Section 5. 2. 1. 6. 3 for details

[c] Benomyl was reported to be positive without activation in strains his G46 and TA 1530 [J. P. Seiler, Experientia 29, 622 (1973)], however, the results could not be confirmed in a more recent study using the same strains [G. Fiscor, S. Bordas and S. J. Stewart, Mutation Res. 51, 151 (1978)].

Table CXIV

Acute Toxicity of Carbamates, Thiocarbamates and Substituted Urea Compounds

Compound	Species and route	LD_{50} (mg/kg)[a]	References
Urethan [Ethyl carbamate]	Mouse, s.c.	2,230, 1,750	(61, 76)
	Hamster, i p.	2,460	(77)
Methyl carbamate	Mouse, oral	6,200	(78)
N-Propyl carbamate	Mouse, s c.	1,300	(76)
Zectran [Mexacarbate]	Rat, oral	37 (M), 25 (F)	(79)
	topical	1,500-2,500	(79)
Propoxur [Baygon]	Mouse, oral	24	(80)
	Rat, oral	83-86	(79)
	i. v.	10.6	(81)
	i. p	44 (M), 33 (F)	(82)
	topical	800-1,000	(83)
	Guinea pig, oral	40	(83)
Carbaryl [Sevin]	Mouse, oral	438, 540	(80, 84)
	Rat, oral	500-850	(79, 85, 86)
	oral	575 (NP), 89 (LP)	(87)
	i. v.	41 9	(81)
	topical	4,000	(79)
	Rabbit, oral	710	(86)
	Cat, oral	150	(88)

Carbofuran [Furadan]	Mouse, oral	2	(80)
	Rat, oral	8.2-14.1	(26)
	topical	10,200	(26)
	inhalation	LC_{50} = 85-133 mg/m^3	(26)
	Guinea pig, inhalation	LC_{50} = 43-74 mg/m^3	(26)
	Dog, oral	19	(26)
	inhalation	LC_{50} = 52 mg/m^3 (exposure time not specified)	(26)
Propham	Mouse, oral	52	(89)
	Rat, oral	1,000-9,000	(90)
Chloropropham	Rat, oral	10,390 (NP), 2,590 (LP)	(87)
Benomyl [Benlate]	Rat, oral	>10,000	(40)
Primicarb	Mouse, oral	107	(83)
	Rat, oral	147	(83)
Aldicarb [Temik]	Mouse, oral	0.3-0.5	(80)
	Rat, oral	0.8 (M), 0.6 (F)	(79)
	topical	3 (M), 2.5 (F)	(79)
Diallate [Avadex]	Rat, oral	395	(91)
	Rabbit, topical	2,000-2,500	(90)
	Dog, oral	510	(83)
Sulfallate	Rat, oral	850	(91)

Sodium diethyldithiocarbamate	Mouse, i.p	1,000	(92)
	Rat, i.p.	1,500	(93)
Ziram [Zinc dimethyldithiocarbamate]	Rat, oral	1,400	(94)
Ferbam [Ferric dimethyldithiocarbamate]	Rat, oral	4,000	(94)
Thiram [TMTD]	Mouse, oral	2,050-2,500	(75)
	Rat, oral	620-640	(79)
	topical	2,000	(79)
	Rabbit, oral	350	(75)
Disulfiram	Mouse, oral	12,000	(95)
	Rat, oral	8,600	(96)
Nabam [Sodium ethylenebisdithiocarbamate]	Rat, oral	395	(97)
Maneb [Manganese ethylenebisdithiocarbamate]	Mouse, oral	4,100	(98)
	Rat, oral	4,500	(98)
Dulcin [4-Ethoxyphenyl urea]	Rat, oral	3,200 (adult)	(99)
Monuron	Rat, oral	2,800 (NP), 950 (LP)	(87)
Diuron	Rat, oral	2,390 (NP); 437 (LP)	(87)

[a] M = male, F = female, NP = normal protein diet (26% casein), LP = protein-deficient diet (3 5% casein).

their mutagenic potential. Various studies of the mutagenicity of carbamates and related compounds have been reported in the past few years. The following discussion focuses only on studies involving the use of the Ames Salmonella test, which is widely regarded as a very useful predictive tool for carcinogenicity (see Suppletory Note 1 for Section 5 2 1 1)

The majority of the mutagenicity studies of carbamates and related compounds used the Salmonella strains TA 100 and 1535 (which detect base-substitution mutagens) and TA 98, 1537 and 1538 (which detect frame-shift mutagens). The major findings of most of these studies are summarized in Table CXV. With the exception of Thiram, none of the compounds displays any significant frame-shift mutagenic activity either in the presence or absence of a liver microsomal activation system. Only the TA 100 and 1535 strains indicated mutagenicity of some of these compounds. With a few notable exceptions (e g , urethan), there is a reasonably good correlation between bacterial mutagenicity and animal carcinogenicity.

Three of the acetylenic N-cyclohexylcarbamates, which have been shown to be potent carcinogens (see Section 5.2 1 6.3.3), also possess potent mutagenic activity Consistently with the direct-acting, alkylating activity of these compounds (25), they do not require metabolic activation for mutagenicity It is interesting to note, however, that Barban and Chlorbupham, which are closely related to the acetylenic (N-cyclohexylcarbamates), do not appear to be mutagenic. The two compounds have been extensively used as pesticides, their carcinogenic potential has yet to be assessed

S-Chloroallyl thiocarbamates (e g., Diallate, Sulfallate, Triallate) represent another class of carbamate compounds that show definite mutagenicity. Although there is some disagreement regarding the mutagenicity of these compounds in the absence of activation, there is little doubt that they are all mutagenic following metabolic activation. The structural moiety common to these compounds is the 2-chloroallyl group, a metabolic intermediate of which is probably responsible for the mutagenic activity Both Diallate and Sulfallate have been unequivocally shown to be carcinogenic In the light of this, it would seem compelling to consider also Triallate to be carcinogenic

The mutagenicity of a series of dithiocarbamate derivatives and related compounds has been tested by Shirasu et al. (45) and Moriya et al (46). An interesting structural requirement for mutagenicity has been noted Thiram, Ferbam, Ziram, sodium dimethyldithiocarbamate, arsenic dimethyldithiocarbamate ——each of which possesses two methyl groups on the amino nitrogens —— have been found mutagenic for strain TA 100 without activation. In contrast, the N, N-diethyl derivatives (Disulfiram, ferric diethyldithiocarbamate, ethyl zimate, sodium diethyldithiocarbamate) and a N-monomethyl derivative (zinc monomethyldithiocarbamate) are all inactive It has been suggested (46) that N, N-disubstitution of the amino nitrogen(s) with methyl groups is essential for the mutagenicity of these compounds It should be pointed out that the mutagenicity of Ferbam and Ziram has not been confirmed by the study of De Lorenzo et al (47) The mutagenicity of Thiram in base-substitution mutants has been confirmed by Zdzienicka et al (48) Interestingly, these

authors (48) also found that the liver metabolic activation system abolishes the base-substitution mutagenic activity, but activates the frame-shift mutagenic activity of the compound.

A number of carbamates and related compounds showed variable results in mutagenicity assays In contrast to its proven carcinogenic activity, urethan has been found by various groups of investigators (41, 49-51) to be inactive in all 5 strains, both in the presence and the absence of a liver activation system, Probably the only positive result was reported by Anderson and Styles (52). However, even in this study only the TA 100 strain was sensitive, whereas the TA 1535 strain was not Inconsistent findings have also been reported in the mutagenicity tests of urethan by detecting point mutation (37, 44), micronucleus formation (41-43), and sister chromatid exchange (35, 38) in mammalian cells. The reason for the variability is not known Benomyl is another compound of some controversy. It was reported to be positive in Salmonella strains his G46 and 1530 (53) and 1535 (54). However, these results could not be confirmed by two subsequent studies (55, 56) Even in the presence of various types of activation systems (including liquid culture assay, host-mediated assay), Benomyl proved to be inactive (55) The disagreement ~~in the findings~~ was attributed to the possible contamination of the chemical used in the earlier studies (55). Disagreement also arose in the case of Monuron. It was found to be inactive by Simmon et al. (57). In a more recent study, it displayed weak but significant mutagenic activity (4) A number of related compounds have also been tested in this study and found to be mutagenic (4)

Teratogenic Effects _The teratogenicity of urethan in the mouse has been extensively investigated by Nomura and coworkers (58-63). Urethan appears to be capable of freely penetrating the placental barrier (62). High incidences of malformations have been observed after the administration to pregnant mice of single, high doses (1.5 or 1 0 g/kg) of urethan during day 8 to day 12 of the gestation period (58, 61) As with other teratogenic agents (see Suppletory Note 2 in Section 5 2.1 1), the induction of malformations in any given organ seems to be totally dependent on the time of differentiation of the organ concerned (58, 61) and, therefore, is determined by the time (during gestation period) of the treatment Malformations of the external appearance are observed with the urethan treatment from day 9 to 12, whereas anomalies of the internal organs occur if urethan is given on day 8 or 9 External malformations frequently observed include tail anomalies (kinky, short, and/or tubercular tail), cleft palate, syndactyly and polydactyly of the limbs. The most affected internal organs are the liver and the lung with interruption of lobulation and sometimes with intrathoracic livers, diaphragmatic hernias and asplenia The dose-response relationship of internal organ anomalies shows a striking non-linearity, the incidence of anomalies drop from 44 to 93% at the dose of 1.5 g/kg to near zero at 1 0 g/kg (58, 59) The teratogenic effects of urethan may be significantly reduced by the administration, within 24 hrs , of caffeine (60) There is some evidence of similarities between the mechanisms of urethan-induced teratogenesis and carcinogenesis (60) The teratogenic effects of urethan on the mouse limbs may also be demonstrated by in vitro organ culture systems (64)

The teratogenicity of urethan has also been shown in Syrian golden hamsters (65). Treatment of pregnant hamsters with urethan on day 8 of gestation induces a variety of malformations (including exencephaly, encephalocele, microcephaly, anophthalmia, microphthalmia, omphalocele, anomalies of extremities) and growth retardation. Nine other related compounds have also been tested by the same authors (65). Of the compounds structurally modified at the carboethoxy end of urethan, n-propyl carbamate is as potent as urethan, β-hydroxyethyl carbamate has marginal activity, whereas allyl- and n-butylcarbamate are inactive Of the compounds modified at the carbamyl end, N-methyl ethyl carbamate is more potent than urethan, diethyl carbonate is as potent, whereas N, N-dimethyl ethyl carbamate is inactive. N-Hydroxyurethan proved to be the most potent teratogen of the group, producing malformations of extremities and anophthalmia in 17 to 44% of the fetuses With a few exceptions, the teratogenicity of the carbamates in the hamster correlates well with their carcinogenicity in the mouse (65).

In addition to the above compounds, the teratogenicity of different carbamate pesticides has been investigated Carbaryl, the most extensively studied carbamate pesticide, has been tested in a variety of animal species, such as the mouse (66, 67), rat (39), hamster (68), guinea pig (68, 69), rabbit (67, 68), dog (70) and monkey (71) Inconsistent findings, due largely to differences in the species, strains and dosages, have been reported The teratogenic action of carbaryl has been demonstrated in the mouse (66), guinea pig (68), rabbit (67) and dog (70). In most cases, high doses, often maternally toxic, are required to exert the teratogenic effect. The positive findings in the guinea pig (68) and rabbit (67) are not in agreement with those of Weil et al (69) and Robens (68),

respectively. Negative findings have been reported with CF-1 mice (67), rat (39), hamster (68), and monkey (71). Another carbamate pesticide, Benomyl, fed at a dietary level of up to 0 5%, was found to be inactive as a teratogen (40). Propham (IPC) has been reported to have a positive but somewhat inconsistent teratogenic activity in the mouse (66)

Among the thiocarbamates and related compounds, Maneb and Zineb have been found teratogenic in the rat, when given at maternally toxic doses However, at doses of 0 5 g/kg (for Maneb) and 1 0 g/kg (for Zineb), they produce no teratogenic effects (72). The teratogenicity of Maneb in the rat has been confirmed by Larsson et al (73), who further demonstrated that the teratogenic effects of Maneb may be reduced by simultaneous treatment with zinc acetate In the NMRI mouse, Maneb has no teratogenic activity (73). Thiram has been found teratogenic in the Syrian golden hamster (68), NMRI (74, 75) and SW mice (74) In the hamster, the malformations include exencephaly, spina bifida, fused ribs, shortened maxila and mandible, and tail and limb anomalies (68). In the mice, cleft palate, wavy ribs, distorted bones of extremities and micrognathy are the most frequently observed effects (74, 75) The NMRI strain is more sensitive to the teratogenic action of Thiram than the SW strain (74) In contrast to Thiram, Disulfiram has no significant teratogenic effects (68), apparently, replacement of the methyl groups with ethyl groups abolishes the teratogenic activity

5 2.1 6 3 Carcinogenicity and Structure-Activity Relationships :

5.2.1 6 3 1 Overview Since the discovery of carcinogenicity of urethan in 1943, close to 100 carbamates and related compounds have been tested for

carcinogenicity. On the basis of the chemical structure and the properties and/or biological functions and uses, these compounds may be classified into the following groups (a) urethan and related compounds, (b) acetylenic carbamates, (c) N-carbamoyl aziridines, (d) carbamate pesticides, (e) thiocarbamate pesticides, and (f) substituted urea compounds. These categories are discussed in the subsequent Sections 5.2.1.6.3.2-5.2.1.6.3.7

Early research efforts were devoted to the elucidation of structure--activity relationships of urethan and related compounds The pioneering studies of Larsen (15, 16) showed that small changes in the chemical structure can have a profound effect on the carcinogenicity of the compound The structural feature which is particularly sensitive to change is the alkyl group, substitution of the ethyl group of urethan by a methyl group completely abolishes carcinogenicity With the exception of N-hydroxylation, N-substitution of urethan also diminishes the carcinogenicity, although to a lesser extent The demonstration by Berenblum et al (17) that N-hydroxy-urethan is almost as potent as urethan, raised the interesting possibility that N-hydroxylation may be a metabolic activating pathway This possibility has been extensively tested by various investigators, in particular by Mirvish and his group, who concluded that N-hydroxylation is not a likely activating pathway (rev 21). One interesting recent finding has been the demonstration of substantially greater carcinogenicity of vinyl carbamate than urethan (107) It underscores the importance of the vinyl group in chemical carcinogenesis and suggests in vivo dehydrogenation as a possible activating pathway

Both acetylenic carbamates and N-carbamoyl aziridines have been discussed separately because of the presence of different highly reactive functional groups in these two classes of compounds From studies with diaryl acetylenic carbamates it appears that the phenyl groups have a crucial role in determining the carcinogenic potency of the compound, whereas changes in the amino group may alter the tissue target specificity. Several N-carbamoyl aziridines are 10-20 times more potent (on a molar basis) than urethan, as a carcinogen The presence of a carbamoyl group renders the aziridine (ethyleneimine) group more reactive by forming, through resonance, an ethyleneimmonium ion which may readily react with nucleophilic sites in cellular macromolecules to initiate carcinogenesis

Ten carbamate pesticides representing various classes such as aryl N-methylcarbamates, alkyl N-aryl carbamates, N, N-dimethylcarbamates, and oxims of carbamates, have been tested for carcinogenicity Among these compounds, β-Sevin (2-naphthyl N-methylcarbamate) is the only compound that has been unequivocally shown to be carcinogenic. This is in sharp contrast to the general lack of carcinogenicity of Carbaryl (1-naphthyl N-methylcarbamate). Apparently, in close analogy to naphthylamines, the introduction of a functional group into the β-position (but not the α-position) of naphthalene confers carcinogenicity to the molecule In addition to β-Sevin, there is some (although not convincing) evidence that Zectran may be carcinogenic

The twenty thiocarbamate pesticides that have been tested for carcinogenicity may be subclassified into 4 groups S-chloroallyl thiocarbamates,

dialkyldithiocarbamates, thiocarbamyl disulfide, and ethylenebisdithiocarbamate. Among these, Diallate and Sulfallate (both S-chloroallyl thiocarbamates) have been unequivocally shown to be carcinogenic On the basis of structural and metabolic considerations, the carcinogenicity of these compounds is due to the S-chloroallyl group. It is expected that this class of compounds may be carcinogenic There is no firm evidence to indicate the carcinogenicity of dialkyldithiocarbamates. Five of the 13 dialkyldithiocarbamates tested in the preliminary NCI bioassay were either shown or suspected to be carcinogenic, three of these five have subsequently been shown to be inactive in the more thorough recent NCI bioassays Only potassium bis(2-hydroxyethyl)dithiocarbamate appears to be carcinogenic in more than one study. No simple structure--activity relationships may be established to associate carcinogenic potential to specific chemical structure It is possible that the metal ion plays a rôle in the biological activity of some of these compounds The two thiocarbamyl disulfides, Thiram and Disulfiram, do not seem to be carcinogenic Among the ethylenebisdithiocarbamates there is some suggestive evidence for the carcinogenicity of Maneb and Zineb, however, it is believed that the carcinogenicity of these may be due to ethylenethiourea present as a metabolite or as impurity

Among five substituted ureas tested, two aliphatic compounds (hydroxyurea and Carbromal) are inactive and two with an aromatic ring (Dulcin and Monuron) show evidence of carcinogenicity, whereas Diuron does not appear to have been adequately tested. Thus, from the limited information available, substituted urea compounds with an aromatic ring are suspect and should be more extensively studied

Urethan has been shown to easily penetrate the placental barrier, the transplacental carcinogenicity of urethan has been reviewed in Section 5.2.1 6.3.8. Urethan is one of the most extensively used experimental carcinogens. A number of carbamates (e.g., Disulfiram) may alter the carcinogenicity of other agents. Many examples of synergism or enhancement of urethan carcinogenesis have been observed.

5.2.1 6.3 2 Urethan and Related Compounds The field of investigations on the carcinogenicity of urethan has been the subject of several comprehensive reviews (11, 21, 108) The major findings of the representative studies in various species and strains of animals are summarized in Tables CXVI and CXVII. Urethan is a multipotential carcinogen in mice, rats, and hamsters Considerable species-, strain- and age-differences have been observed The route of administration does not seem to affect the organotropism of tumorigenesis to any great extent, although the doses and schedule of treatment may play some role.

Tables CXVI & CXVII

The carcinogenicity of urethan has been tested in over 30 different strains and substrains of mice In most of these strains, the lung is the most affected organ. The induction of lung tumors may occur irrespective of whether urethan is administered by oral, i p., i v , s c , topical or inhalational route In addition to the lung, the hematopoietic system and the liver are often affected, especially in younger mice. Other carcinogenicity targets in some specific strains include the mammary gland, Harderian gland, forestomach, fat pad, intestines, skin and salivary gland

Table CXVI

Carcinogenicity of Urethan in Adult Animals

Species and strain	Route	Principal organs affected	References
Mouse, A	oral, i.p or i.v	Lung	(263)
Mouse, A/Jax	i.p.	Lung	(116)
Mouse, AK	i.p.	Lung	(110)
Mouse, AKR	i.p.	Hematopoietic system	(264)
Mouse, Balb/c	i.p.	Lung	(199, 265)
Mouse, Bagg	i.p.	Lung	(109, 110)
Mouse, BLH	inhalation	Lung	(266)
Mouse, (Bagg X DBA)F_1	i.p.	Lung	(109)
Mouse, C	s.c.	Lung	(267)
Mouse, C3H	oral	Lung, hematopoietic system, fat pad	(13)
	i.p.	Mammary gland, lung	(10)
	topical	Mammary gland, lung, fat pad	(10)
Mouse, C57	i.p.	Lung	(110)
	i.p.	Liver, intestines	(268)

Mouse, C57BL	inhalation	Lung	(266)
Mouse, C58	i. p.	Hematopoietic system, liver, intestines	(264)
Mouse, (C57 X A/J)F_1	oral	Lung	(188)
Mouse, (C57 X C3H)F_1	i. p. or topical	Lung, mammary gland, fat pad, Harderian gland	(10, 12)
	i. p.	Lung, Harderian gland, liver	(189)
Mouse, CBA	i. p.	Lung	(109)
Mouse, CTM	oral	Lung, hematopoietic system, mammary gland, liver, Harderian gland	(270, 271)
Mouse, Db	i. p.	Lung, hematopoietic system, liver	(265)
Mouse, DBA	oral, i p. or topical	Lung, mammary gland, fat pad	(10, 13)
Mouse, DBA/2eBDE	i. p. or topical	Liver, hematopoietic system, lung, fat pad, Harderian gland	(269)
Mouse, dd	oral	Lung	(272)
Mouse, FA	i. p.	None	(109)
Mouse, FB	i. p.	Lung	(109)

Mouse, Hall	s.c.	Lung, liver, hematopoietic system, skin	(273)
Mouse, NH	i.p.	Lung	(109)
Mouse, NMRI	inhalation	Lung	(266)
Mouse, NZO/Bl	i.p.	Skin	(111)
Mouse, Stock albino 'S'	topical	None	(8)
Mouse, Strong A	i.p.	Lung	(109)
Mouse, Swiss	oral	Lung, hematopoietic system	(186, 274)
	oral	Forestomach	(9)
	i.p	Lung	(109)
	s.c.	Lung	(267)
Mouse, "White-footed"	i.p.	None	(110)
Mouse, Zb	i.p.	Lung, hematopoietic system, liver, mammary gland	(265)
Rat, MRC	i.p.	Nervous system, thyroid gland, liver	(126)
Rat, Sprague	oral	Liver, adrenal cortex, hematopoietic system, mammary gland	(125)

Rat, Sprague-Dawley	oral or i p.	Mammary gland, ear duct (Zymbal's gland), hematopoietic system, kidney	(14)
Hamster, Syrian golden	oral	Skin, forestomach, intestines, lung, mammary gland, liver	(275, 276, 277)
	oral or topical	Skin, mammary gland, ovary	(278-281)
	s. c.	Skin, forestomach, intestines	(134, 282)
Hamster, European	i. p.	Subcutaneous and peritoneal tissues, (with lower incidence liver, lung, adrenal gland, nasal cavity, kidney, forestomach)	(77)
Guinea pig	oral	None	(136)
Chicken (Brown Leghorn)	oral or i p.	None	(136)

Carcinogenicity of Urethan in Newborn or Pre-weanling Animals

Species and strain	Route	Principal organs affected	Reference
Mouse, AKR	s.c.	Hematopoietic system	(192)
Mouse, Balb/c	s.c.	Lung	(112)
Mouse, C3Hf/Lw	s.c.	Liver, lung	(112)
Mouse, C57Bl	s.c.	Hematopoietic system	(283)
	i.p.	Lung, hematopoietic system	(284)
Mouse, (C57xA/J)F_1	i.p.	Lung, liver, hematopoietic system	(188, 196)
Mouse, (C57xC3H)F_1	i.p.	Liver, lung, hematopoietic system, Harderian gland, ovary	(113, 189, 193, 198)
Mouse, Charles River CD-1	i.p.	Hematopoietic system, liver, lung	(285)
Mouse, DBA/f	s.c.	Liver, lung	(112)
Mouse, dd	s c.	Hematopoietic system	(201, 202)
Mouse, dd/I	s.c.	Hematopoietic system, lung, Harderian gland, liver	(287)
Mouse, Swiss	s.c.	Hematopoietic system	(192, 286)
	s.c.	Liver	(190)
	s.c.	Lung	(186)
Mouse, XVII/G	s.c.	Lung, hematopoietic system, salivary gland, spleen	(171)
Rat, August hooded	s c.	Eye	(130, 131)

Rat, MRC	i.p.	Nervous system, liver	(126)
	i.p.	Liver, pituitary gland, uterus, nervous system, mammary gland, and other various sites	(128, 129)
Hamster, Syrian golden	s.c.	None (single low dose)	(132)
Hamster, Syrian golden	s.c.	Adrenal cortex, liver, forestomach, pancreas	(133)
	s.c.	Forestomach, skin, intestine, thyroid gland	(134)
Hamster, Syrian white	i.p.	Skin, stomach, liver, kidney	(135)
Guinea pig, Hartley albino	s.c.	Lung, ovary	(137)

The considerable strain differences in the susceptibility of mice to the carcinogenic action of urethan may be best illustrated by the studies of Shapiro and Kirschbaum (109) and Gross et al (110) In the former study (109), mice of 8 different strains were given weekly i. p. injections of urethan (1 g/kg) for 6 weeks, starting at the age of 10 weeks, and were killed 6-9 months later The lung tumor incidence and multiplicity (average number of nodules/mouse) were, in decreasing order of susceptibility, as follows Strong A, 100%, 15 nodules, Bagg, 100%, 10, (Bagg X DBA)F_1, 100%, 8, NH, 100%, 7, CBA, 96%, 4, DBA, 16%, 2, FB, 12%, 1, and FA, 0%, 0. In the study of Gross et al (110), the lung tumor incidence in ~~the~~ six different strains was as follows Swiss albino, 100% (36/36), Bagg albino, 100% (16/16), C3H, 95% (189/199), AK, 71% (17/24), C57 BL, 70% (33/47), "white-footed" field mice, 0% (0/79) In addition to FA strain and "white-footed" field mice, stock albino 'S' mice were reported to be resistant to the carcinogenic action of urethan applied topically (8) On the other hand, Bielschowsky et al (111) noted that an unusual strain (NZO/Bl) of mice developed skin tumors after receiving i p administration of urethan alone, in all other strains, the application of a promotor, such as croton oil, is needed for the expression of skin carcinogenicity of urethan Strain differences in the susceptibility to liver carcinogenesis by urethan have also been noted, Trainin et al (112) reported that after administration of 2 mg urethan subcutaneously shortly after birth, 100% of C3Hf/Lw, 86% of male DBAf but none of BALB/c mice developed hepatomas. However, the lung tumor incidence was highest in BALB/c mice (76%) followed by DBAf (34%) and C3Hf (17%).

Newborn and infant mice are more susceptible to the carcinogenic action of urethan than are adults Thus, whereas tumors of the hematopoietic system and liver rarely develop in adult mice (Table CXVI), such tumors are readily observed in mice treated with urethan at newborn age (Table CXVII). Susceptibility generally decreases as the animal ages (this topic will be discussed in more detail in Section 5.2.1.6 3 9)

Several investigators have noted that the dosage and schedule of treatment may affect the carcinogenicity of urethan For example, Vesselinovitch and Mihailovich (113) observed that continuous treatment with urethan, starting at the newborn stage, is significantly more efficient in inducing leukemia than if such treatment is interrupted for various periods of time. About 32% of (C57 X C3H)F_1 mice developed leukemia after receiving 6 doses of urethan at 3-day intervals starting on the 1st day of their life. The incidence dropped to 13% and 4% if the interval between the 3rd and 4th injections was extended to 9 or 21 days, respectively. It is possible that this is due to the effect of age rather than to specific dosage effect In an experiment by Gubareff [as reported by Shimkin et al. (114)]distribution of a given dose of urethan into several smaller doses caused either an increase or a decrease in the tumor yield, the direction of change depended on the number of the doses and the time interval of spacing However, this finding was only partially confirmed by Shimkin et al (114) and White et al (115), who noted only a decrease in tumor yield upon fractionation and spacing of a given dose of urethan Age was not considered to be a factor, since the decreasing effect of fractionation occurred in mice of different ages ($4\frac{1}{2}$, $6\frac{1}{2}$ and $8\frac{1}{2}$ weeks) (116)

As mentioned earlier, the route of administration appears to have very little effect on the organotropism of tumorigenicity of urethan This also holds for the initiation of skin tumors by urethan. Whether administered topically (7, 8, 117), orally, intraperitoneally or subcutaneously (9, 118-122), urethan induced skin tumors after promotion by promotors, such as croton oil. The promotion with croton oil may be delayed for 8 weeks with no significant change in tumor yield (9), a delay of 24-30 weeks may result in a decrease of about 50% in tumor incidence (120, 123) An additional application of croton oil shortly before urethan initiation may substantially enhance the skin carcinogenicity of urethan (120, 122) Goerttler and Lohrke (124) have recently reported an interesting finding that urethan-induced skin tumor initiation may occur transplacentally and may be promoted postnatally by 12-O-tetradecanoylphorbol-13--acetate, the active ingredient of croton oil (see Section 5 2 1 6 3 8)

The multipotential carcinogenicity of urethan in the rat has been well established and considerable strain differences have also been observed In young adult Sprague-Dawley rats the principal tissues affected are the mammary gland, the ear duct (Zymbal's gland), the hematopoietic system and the kidney (14), the overall tumor incidence was as high as 85% In female Sprague rats (Gif-sur-Yvette strain), treated with urethan from the age of 4-5 months, 82% developed tumors predominantly in the liver, adrenal cortex, hematopoietic system and mammary gland (125) In the study of Kommineni et al (126) using young adult MRC rats, tumors were mainly found in the neural tissues (neurilemmomas), thyroid gland and liver In a study designed to investigate the synergistic

effect between urethan and X-ray, three different strains of rats were used, the average number of mammary tumors (induced by urethan alone) per female rat was 2.3 in Sprague-Dawley rats (control 0.4), 1.8 in Long-Evans rats (no control), and 0 1 in Collip rats (control 0 02) suggesting different susceptibility of the tissue to the carcinogenic action of urethan among these strains (127).

The carcinogenic effects of urethan in newborn rats may be quite different from those in adults. In the study of Komminen et al (126) mentioned above, much higher incidences of neurilemmomas and liver tumors were observed in rats treated at newborn age. Notably, the thyroid gland was not affected in these rats. It was suggested that the "functional status" of the tissue may play a significant role in determining the susceptibility of the tissue to carcinogenesis In the study of Vesselinovitch and Mihailovich (128, 129), newborn MRC rats responded to urethan treatment with the development of a variety of tumors in the liver, pituitary gland, uterus, nervous system, mammary gland and various other sites In newborn August hooded rats, s c injections of urethan led to the induction of an unusual type of tumors (melanoma of the eye). The iris, ciliary body and/or choroid were affected No tumors attributable to the administration of urethan occurred at other sites (130, 131) It is not known whether this peculiar target tissue is specific to the newborn of this strain

The carcinogenicity of urethan has also been investigated in different strains of hamsters In adult Syrian golden hamsters, the induction of melanotic tumors of the skin appears to be the most predominant carcinogenic effect of

urethan Skin tumors developed irrespective of whether urethan was given orally, i.p. or s.c (see Table CXVI) The induction of papillomas and carcinomas of the forestomach was also frequently observed after oral or s.c administration Other susceptible tissues are the mammary gland, ovary, intestines, lung and liver Thus, urethan is also a multipotential carcinogen in the hamster. In the wild European hamster, up to 80% of the animals developed tumors after receiving i p injections of urethan (77) Most of these tumors were subcutaneous, intraperitoneal, and subperitoneal fibrosarcomas About 10% of the treated animals developed adrenal pheochromocytomas, and in a few cases tumors of the respiratory system, liver and forestomach also occurred

Newborn hamsters exhibited a somewhat different carcinogenic response to urethan. A single s.c dose of 150 µg was ineffective in inducing tumors, probably because the dose was too low (132) Six weekly s.c. injections of 1 g/kg starting at the age of 7 days led to the induction of adrenal cortical tumors in 25-30% of the animals, a few tumors also occurred in the liver, forestomach and pancreas (133) Toth (134) compared the carcinogenic response of newborn and adult Syrian golden hamsters to urethan More intestinal tumors developed in newborns, whereas the reverse was true for papillomas of the forestomach. The incidence of other types of tumors was not significantly affected by age. Vesselinovitch et al (135) administered an i p dose of 0 5 g/kg urethan to newborn Syrian white hamsters and continued injections at 3-day intervals until a total dose of 2.5 g/kg was reached About 46% of the males and 27% of the females developed tumors, mostly malignant melanomas of the skin

In addition to mice, rats and hamsters, the carcinogenicity of urethan was tested in adult guinea pigs and chickens (136) These latter two species proved to be resistant to the carcinogenic effect of urethan The refractoriness of the guinea pig may be, however, reduced if urethan is given at neonatal age. Toth (137) reported that 33-35% of Hartley albino guinea pigs developed tumors, mostly in the lung and ovary, after receiving 5 s.c doses of 1 g/kg urethan starting within 24 hours after birth.

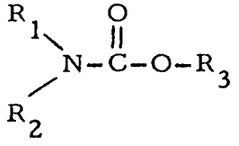
The elucidation of the relationships between the chemical structure and carcinogenicity of urethan and related compounds has been of great interest for several decades Larsen (15, 16), Berenblum et al (17), Shimkin et al (138), and Pound and Lawson (139, 140) have made significant contributions in synthesizing and testing a variety of urethan analogs. Most of these studies were carried out in the mouse using lung tumor induction and skin tumor initiation as the indicators of carcinogenicity The results of these studies are summarized in Table CXVIII. Compounds are assigned arbitrary ratings for the purpose of comparison It is salient from the table that minor modification of the chemical structure can have profound effect on the carcinogenicity of the compound. In general, modification of the ester group appears to bring about a more dramatic effect on the carcinogenicity, whereas substitution at the amino group produces a more gradual change

Table CXVIII

The effect of modification of the ester group may be illustrated by compounds listed in group (A) Table CXVIII Substitution of the ethyl group by any other alkyl group either greatly diminishes or completely abolishes the

Table CXVIII

Relative Carcinogenic Potency of Urethan and Its Structural Analogs in the Mouse[a]

Compound	Structure	Pulmonary tumorigenicity		Skin tumor-initiating activity		Other
						
Urethan (ethyl carbamate)	$R_1 = R_2 = H-$, $R_3 = C_2H_5-$	+++	(15-17, 107, 138, 139, 226, 273, 288)	+++	(17, 139, 107, 273, 288)	Liver +++ (273) Mammary +++ (141) Lymphosarcoma +++ (284)
(A) Modification of the Ester Group	($R_1 = R_2 = H-$)					
Methyl carbamate	$R_3 = CH_3-$	-	(15, 138, 273)	-	(273)	Liver - (273)
n-Propyl carbamate	$R_3 = CH_3CH_2CH_2-$	+ ± -	(15, 17) (138) (273)	+ -	(17) (273)	Liver - (273)
iso-Propyl carbamate	$R_3 = (CH_3)_2CH-$	+	(15, 138, 273)	-	(273)	Liver + (273)
n-Butyl carbamate	$R_3 = CH_3(CH_2)_2CH_2-$	-	(15, 138, 226, 273)	-	(273)	Liver ± (273) Mammary +++ (141)
sec-Butyl carbamate	$R_3 = CH_3CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H-$	-	(138)	n. t.		

iso-Amyl carbamate	$R_3 = (CH_3)_2CHCH_2CH_2-$	-	(15, 226)	n. t.		
n-Hexyl carbamate	$R_3 = CH_3(CH_2)_4CH_2-$	-	(138)	n. t.		
Vinyl carbamate	$R_3 = CH_2{=}CH-$	++++	(107)	++++	(107)	
Allyl carbamate	$R_3 = CH_2{=}CHCH_2-$	- ++	(17) (138)	+	(17)	
Methylallyl carbamate	$R_3 = CH_2{=}C(CH_3)CH_2-$	-	(138)	n. t.		
Phenyl carbamate	$R_3 = C_6H_5-$	-	(138)	n. t.		
Benzyl carbamate	$R_3 = C_6H_5CH_2-$	-	(138)	n. t.		
β-Hydroxyethyl carbamate	$R_3 = HOCH_2CH_2-$	-	(17, 138)	±	(17)	
β-Hydroxypropyl carbamate	$R_3 = CH_3-CH(OH)-CH_2-$	-	(138)	n. t.		
β-Aminoethyl carbamate	$R_3 = H_2NCH_2CH_2-$	-	(17)	-	(17)	
β-Chloroethyl carbamate	$R_3 = ClCH_2CH_2-$	-	(15, 138)	n. t.		
β,β,β-Trichloroethyl carbamate	$R_3 = Cl_3CCH_2-$	+	(15)	n. t.		
(B) Modification of the Amino Group	($R_3 = C_2C_5-$)					
N-Methyl ethyl carbamate	$R_1 = CH_3-$, $R_2 = H-$	++	(16, 17, 273)	++	(17, 273)	Liver ++ (273)
N, N-Dimethyl ethyl carbamate	$R_1 = R_2 = CH_3-$	+ -	(16) (17)	-	(17)	

N-Ethyl ethyl carbamate	$R_1 = C_2H_5-$; $R_2 = H-$	+ (16) ++ (273)	++ (273)	Liver ++ (273)
N, N-Diethyl ethyl carbamate	$R_1 = R_2 = C_2H_5-$	+ (16)	n. t.	
N-n-Propyl ethyl carbamate	$R_1 = CH_3CH_2CH_2-$, $R_2 = H-$	+ (16) ++ (273)	+ (273)	Liver ++ (273)
N, N-Di-n-propyl ethyl carbamate	$R_1 = R_2 = CH_3CH_2CH_2-$	+ (16)	n. t.	
N-n-Butyl ethyl carbamate	$R_1 = CH_3CH_2CH_2CH_2-$, $R_2 = H-$	+ (16)	n. t.	
N, N-Di-n-butyl ethyl carbamate	$R_1 = R_2 = CH_3CH_2CH_2CH_2-$	+ (16)	n. t.	
N, N-Diphenyl ethyl carbamate	$R_1 = R_2 = C_6H_5-$	- (16)	n. t.	
N-Hydroxy ethyl carbamate	$R_1 = HO-$, $R_2 = H-$	++ (17, 288) +++ (284)	++ (17, 284) +++ (139)	Lymphosarcoma +++ (284)
N-Hydroxy-N-methyl ethyl carbamate	$R_1 = HO-$, $R_2 = CH_3-$	n t	- (139)	
N-Acetyl ethyl carbamate	$R_1 = CH_3CO-$, $R_2 = H-$	++ (138)	n. t.	
N-Cyanoacetyl ethyl carbamate	$R_1 = N\equiv CCH_2CO-$, $R_2 = H-$	++ (138)	n. t.	
Urethan phosphate	$R_1 = PO(OH)_2-$, $R_2 = H-$	± (17)	± (17)	
Carboethoxyglycine	$R_1 = HOOCCH_2-$, $R_2 = H-$	- (17)	- (17)	

Table CXVIII, continued

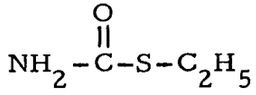
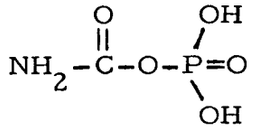
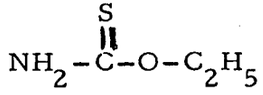
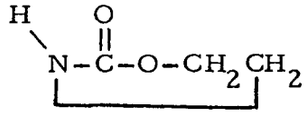
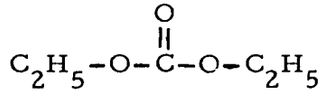
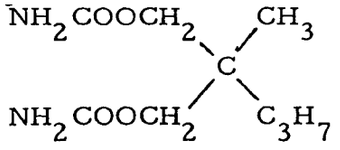
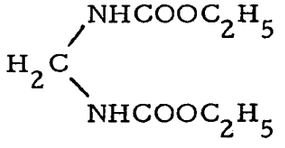
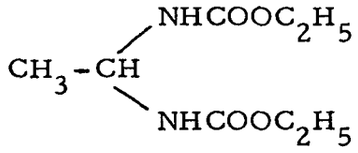
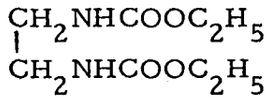
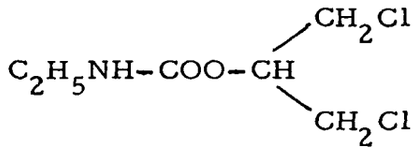
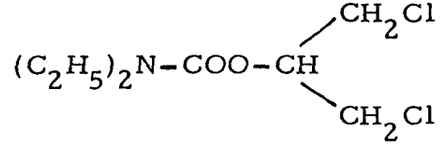
(C) Other Structurally Related Compounds					
Thiourethan		-	(17)	±	(17)
Carbamyl phosphate		-	(17)	±	(17)
Xanthogenamide		-	(17)	±	(17)
Oxazolidone		-	(17)	+	(17)
Diethyl carbonate		-	(17)	+	(17)
2-Methyl-2-n-propyl 1,3-propanediol dicarbamate		-	(17)	-	(17)
Methylene diurethan		+++	(16)	n. t.	

Table CXVIII, continued

Ethylidene diurethan		+++ (16)	n. t.
Ethylene diurethan		++ (16)	n. t.
N-Ethyl 1, 3-dichloro-isopropyl carbamate		++++ (142)	n. t.
N, N-Diethyl 1, 3-dichloro-isopropyl carbamate		++++ (142)	n. t.

[a] Relative potency ++++ = considered stronger than urethan, +++ = of comparable activity to urethan, ++ = less potent than urethan, + = slight carcinogenic activity, ± = questionable carcinogenic activity, - = inactive.

carcinogenicity. This trend is observed in the induction of lung tumors and initiation of skin tumors, as well as production of liver tumors. Only n-propyl and isopropyl carbamates exhibit some, although often not convincing, evidence of carcinogenicity. The induction of mammary gland tumors may, however, be an exception to the general observation of lower carcinogenicity of alkyl carbamates other than urethan n-Butyl carbamate was found to be as potent as urethan in inducing mammary tumors in C3H mice (141)

Substitution of the ethyl group with a vinyl yields a highly carcinogenic compound Dahl et al (107) have recently shown that vinyl carbamate is about 10-50 times more potent than urethan in inducing lung tumors and initiating skin tumors. The potent carcinogenicity of vinyl carbamate is not due to the presence of the double bond alone. Allyl, methylallyl, phenyl and benzyl carbamates have all been shown to be either inactive or much less active than urethan Apparently, a two-carbon moiety may be the optimal size for carcinogenic activity in carbamates. Vinyl carbamate has been suggested to be a possible active metabolite of urethan through in vivo dehydrogenation (see Section 5 2.1 6 4) It has been postulated (107) that the electrophilic epoxide formed from the vinyl group may be the "ultimate carcinogen" structural feature Binding studies are in support of the view that the ethyl group of urethan is involved in the initial event in carcinogenesis (see Section 5 2 1 6 4)

Introduction of a polar (hydroxy or amino) group into the ethyl moiety in urethan completely abolishes carcinogenicity Such modification probably enhances urinary excretion and/or prevents metabolic activation β-Chloroethyl carbamate is inactive, whereas β,β,β-trichloroethyl carbamate has slight carcinogenic activity

The effect of structural modification of the amino group is illustrated by compounds in group (B) of Table CXVIII. N-Monosubstitution with alkyl groups generally reduces the carcinogenicity of urethan The lowering effect on lung tumorigenicity and skin tumor initiating activity appears to be dependent on the size of the alkyl group, the decrease being greater with larger alkyl groups. The ability of N-alkyl ethyl carbamates to induce liver tumors, however, does not seem to vary among the derivatives tested. N, N-Disubstitution with alkyl groups further diminishes or abolishes the carcinogenicity of urethan.

N-Hydroxyurethan is the only N-substituted compound that displays comparable or slightly less carcinogenicity than urethan itself This observation, coupled with the knowledge that N-hydroxyurethan is chemically more reactive than urethan, led some investigators to propose N-hydroxylation as a possible metabolic activating pathway of urethan (see Section 5 2 1 6.4) In contrast to N-hydroxyurethan, N-hydroxy-N-methyl-ethyl carbamate is completely devoid of skin tumor initiating activity, suggesting that di-substitution abolishes carcinogenicity. N-Substitution with acetyl or cyanoacetyl group decreases the carcinogenicity, whereas substitution with a phosphate or carboxyethyl group completely abolishes activity.

The compounds listed in group (C) of Table CXVIII substantiate the view that the ester group of urethan probably plays a significant role in the carcinogenicity of the compound Compounds with modified ester groups (such as thiourethan, carbamyl phosphate, xanthogenamide, oxazolidone and 2-methyl-2--n-propyl 1, 3-propanediol-dicarbamate) are all inactive as lung carcinogens,

and marginally active as skin-tumor initiators It is interesting to point out that replacement by sulfur of either the carbonyl or ethereal oxygen of urethan yields completely inactive compounds. In contrast to the above, compounds with a slightly modified amino group (e g., methylene diurethan, ethylidene diurethan, ethylene diurethan) display carcinogenicity comparable to or slightly less than urethan. Nonetheless, the amino group is required as can be shown by the lack of carcinogenicity of diethylcarbonate Two chlorinated derivatives of urethan (N-ethyl 1, 3-dichloroisopropyl carbamate and N, N-diethyl 1, 3-dichloroisopropyl carbamate) were claimed to be more potent carcinogens than urethan (142), however, the details of the study are not available On the basis of the known structure-activity relationships of urethan derivatives, the supposedly higher activity of the two compounds remains questionable.

5 2 1.6 3.3 Acetylenic Carbamates Diaryl acetylenic carbamates were originally developed as a new class of potential antineoplastic agents (143) A 90-day subacute toxicity study of 1, 1-diphenyl-2-propynyl N-cyclohexyl-carbamate [compound (1) in Table CXIX] revealed the remarkable carcinogenicity of the compound (144) Harlan rats fed diets containing 0.1, 0 25 or 0 5% of the compound developed lymphoblastoma affecting mainly the spleen, liver, adrenals, and lung In a subsequent chronic study (475 days duration), using dietary levels of 0 025 to 0.1%, carcinomas of the mammary gland, duodenum, Zymbal's gland (in ear duct) and liver were detected Daily s c injections of 12 5 to 50 mg/kg of the compound for 11 weeks induced in this decreasing order of incidence local sarcomas (38/60 rats), mammary tumors (24/60), lymphoblastoma

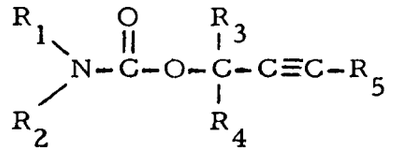
(5/60) and carcinomas of the Zymbal's gland (3/60) Swiss albino and AK mice and Mongolian gerbils also developed lymphoblastoma after ingestion of the compound (144).

Intrigued by the above finding, Harris and associates (145-147), extended their study to nine other compounds of the same class The results are summarized in Table CXIX. Interesting structure-activity relationships may be derived from these data Both the carcinogenic potency and organotropism of the compound are dependent on the nature of the substituent groups The R_3 and R_4 groups seem to play an important role in determining the carcinogenic potency of the compound The replacement of one of the phenyl groups of compound (i) with a methyl group [giving rise to compound (vi)] completely abolished the carcinogenicity in male rats and diminished the potency in female rats (146) Introduction of electron-donating groups (e g , methyl) into the phenyl ring tends to decrease the carcinogenicity, whereas ring substitution with electronegative groups (e g , chlorine, fluorine) has the opposite effect Thus, compound (vii) was found to be a weaker carcinogen than compound (i) (146), whereas compounds (iii), (viii) and (ix) appeared to be more potent Compounds (viii) and (ix) were so potent that dietary levels as low as 0 05-0 25% and 0.01-0 05% were sufficient to induce tumors in as early as 64 and 54 days, respectively (145) The amino group seems to determine the carcinogenicity target of the compound, N, N-disubstitution shifts the organotropism as well as enhances the potency Compound (v) induces a high incidence of hepatocarcinomas in rats receiving diets containing only 0 005% of the compound (146)

Table CXIX

Carcinogenicity of Acetylenic Carbamates in the Rat after Oral Administration

Structure



Compound	R_1	R_2	R_3	R_4	R_5	Principal carcinogenicity targets	Reference
(i) 1,1-Diphenyl-2-propynyl-N-cyclohexylcarbamate	C_6H_{11}-	H-	C_6H_5-	C_6H_5-	H-	Hematopoietic system, mammary gland, intestine, ear duct, (Zymbal's gland), liver	(144, 146)
(ii) 1,1-Diphenyl-2-propynylcarbamate	H-	H-	C_6H_5-	C_6H_5-	H-	Mammary gland (females), intestine (males)	(146)
(iii) 1-(4-Chlorophenyl)-1-phenyl-2-propynyl carbamate	H-	H-	Cl-C_6H_4-	C_6H_5-	H-	Mammary gland (females), intestine (males), palate, brain	(147)
(iv) 1,1-Diphenyl-2-propynyl-N-ethylcarbamate	C_2H_5-	H-	C_6H_5-	C_6H_5-	H-	Hematopoietic system, mammary gland, intestine, ear duct, liver	(146)
(v) 1,1-Diphenyl-2-propynyl-N,N-dimethylcarbamate	CH_3-	CH_3-	C_6H_5-	C_6H_5-	H-	Liver	(146)
(vi) 1-Phenyl-1-methyl-2-propynyl N-cyclohexylcarbamate	C_6H_{11}-	H-	CH_3-	C_6H_5-	H-	Mammary gland (females), none (males)	(146)

Table CXIX, continued

(vii)	1-Phenyl-1-(3,4-xylyl)-2-propynyl N-cyclohexylcarbamate	C_6H_{11}-	H-	$(CH_3)_2C_6H_3$-	C_6H_5-	H-	Intestines (moderate), liver (weak)	(146)
(viii)	1,1-Bis-(4-fluorophenyl)-2-propynyl N-cycloheptylcarbamate	C_7H_{13}-	H-	F-C_6H_4-	F-C_6H_4-	H-	Hematopoietic system, mammary gland	(145)
(ix)	1,1-Bis-(4-fluorophenyl)-2-propynyl N-cyclooctylcarbamate	C_8H_{15}-	H-	F-C_6H_4-	F-C_6H_4-	H-	Hematopoetic system, intestines (Harlan rats)	(145)
							Ear duct, intestines, hematopoietic system (F344 males)	(148)
							Ear duct, mammary gland, intestines, hematopoietic system (Sprague-Dawley rats)	(148)
(x)	1,1-Diphenyl-2-butynyl-N-cyclohexylcarbamate	C_6H_{11}-	H-	C_6H_5-	C_6H_5-	CH_3-	Mammary gland, intestines, liver (females), liver, intestines (in a few males)	(145)

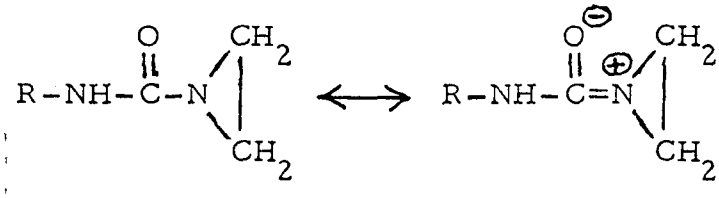
Acetylenic carbamates with free amino groups [compounds (ii) and (iii)] induced mammary tumors in female and intestinal tumors in male rats (146, 147) Most N-monosubstituted compounds have similar organotropism, affecting mainly the hematopoietic system, the mammary gland and the intestines.

An interesting strain-difference in the carcinogenicity of compound (ix) has been reported by Weisburger et al. (148) Male F344 rats given compound (ix) developed squamous cell carcinomas of the ear duct, carcinomas of small intestine and lymphomas with about the same incidences However, male Sprague--Dawley rats developed a greater number of ear duct tumors than at any other sites. There was an unusually high incidence of mammary adenocarcinomas, 22/75 males developed such tumors (148) These results are quite different from those in the study of Harris and associates using Harlan rats which, upon receiving compound (ix), developed lymphomas with a 100% incidence. Both the ear duct and the mammary glands of the Harlan rats were not significantly affected by the compound (145)

Two acetylenic carbamate pesticides have recently been gaining increasingly important roles in crop protection These two compounds, Barban (R_1 = m-Cl-C_6H_4-, $R_2 = R_3 = R_4$ = H-, R_5 = $ClCH_2$-) and Chlorobupham (R_1 = m-Cl-C_6H_4-, $R_2 = R_3$ = H-, R_4 = CH_3-, R_5 = H-) are similar in structure to the carcinogenic compounds discussed above (Table CXIX) and are, therefore, suspect There is no information on the carcinogenicity of Barban and Chlorobupham, Salmonella tests have, however, been consistently negative (see Table CXV) It is possible that replacement of the two phenyl groups greatly

reduces or abolishes the potential carcinogenicity of the compounds. Nevertheless, in view of the wide use of these compounds, carcinogenicity tests are urgently called for to ensure that they would not pose any health hazard to humans.

5.2 1.6.3.4 N-Carbamoyl Aziridines Like the highly reactive acetylenic carbamates, N-carbamoyl aziridines constitute a special class of carbamoyl compounds with a highly reactive functional group —— aziridine (ethyleneimine) (see also Section 5 2 1 1 4). N-Carbamoyl aziridines are probably more reactive than unsubstituted aziridines toward biological nucleophiles, possibly because of the resonance structures

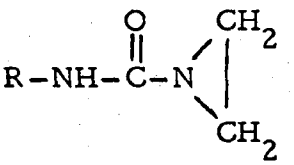


The carcinogenicity of eight N-carbamoyl aziridines has been tested by Shimkin et al. (138) using strain A/He mice The relative potencies in inducing lung tumors are summarized in Table CXX. Several of the compounds are, on a molar basis, much more active than urethan, for example, 3,4-dichlorophenyl-N-carbamoyl aziridine was estimated to be 23 times more potent than urethan. The nature of the substituent group (R) in the carbamoyl moiety plays a crucial role in determining the carcinogenicity of the compound Saturation of the phenyl ring greatly reduces carcinogenicity, the relative potency of cyclohexyl-N-carbamoyl aziridine was more than 7 times less than that of phenyl--N-carbamoyl aziridine (138) However, there is no simple relationship

Tab CXX

Table CXX

Relative Carcinogenic Potency of N-Carbamoyl Aziridines in the Induction of Pulmonary Tumors in Strain A/He Mice.[a]



Compound	R group	Relative potency[b]
3,4-Dichlorophenyl-N-carbamoyl aziridine	3,4-diCl-C_6H_3-	117
3-Chlorophenyl-N-carbamoyl aziridine	3-Cl-C_6H_4-	56
Phenyl-N-carbamoyl aziridine	C_6H_5-	51
Cyclohexyl-N-carbamoyl aziridine	Cyclohexyl-	7
4-Methoxyphenyl-N-carbamoyl aziridine	4-CH_3O-C_6H_4-	±
p-Tolyl-N-carbamoyl aziridine	4-CH_3-C_6H_4-	±
2-Ethoxyphenyl-N-carbamoyl aziridine	2-C_2H_5O-C_6H_4-	—
4-Fluorophenyl-N-carbamoyl aziridine	4-F-C_6H_4-	—

[a] Summarized from the data of M. B. Shimkin, R. Wieder, M. McDonough, L. Fishbein, and D. Swern, Cancer Res 29, 2184 (1969).

[b] For comparison, urethan was assigned a relative potency of 5.0 in the same study. The relative potencies are designated ± = marginal and — = inactive The compounds were administered intraperitoneally

between ring substitution and carcinogenicity Ring substitution with electron--donating groups (methyl, methoxy, ethoxy) yields compounds that are either equivocal or inactive, whereas substitution with the electron-attracting chlorine atoms greatly enhances the carcinogenicity. However, ring substitution with the more electronegative fluorine completely abolishes activity. It is possible that ring substitution with fluorine gives rise to compounds that are too reactive to reach target macromolecules in the cell.

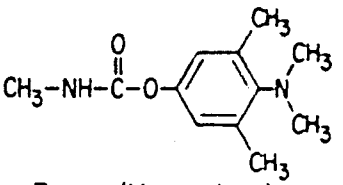
5.2.1.6 3.5 Carbamate Pesticides Carbamates have increasingly been used as pesticides in recent years, the annual consumption of some of these compounds is in excess of a million pounds (see Section 5 2.1 6 5). The increasingly prevalent use of carbamates is likely to continue as more and more organochlorine pesticides are banned. Despite extensive acute toxicity studies of carbamate pesticides, only ten such compounds have thus far been tested for carcinogenicity The structural formulas of these compounds are depicted in Table CXXI and the carcinogenicity testing results are summarized in Table CXXII

Among the ten compounds tested, five belong in the group of aryl N-methylcarbamates. Zectran (Mexacarbate) is a phenyl N-methylcarbamate with two methyl groups and one dimethylamino group attached to the ring. It was first suspected to be carcinogenic in a preliminary NCI bioassay (18, 19) Oral administration of the compound (4 6 mg/kg) for 77 weeks to B6C3F$_1$ mice, starting at the age of 7 days, led to a slight but significant increase in the incidence of lung tumors in both sexes and in the incidence of hepatomas in male mice In contrast to B6C3F$_1$ mice, no significant carcinogenic effects in

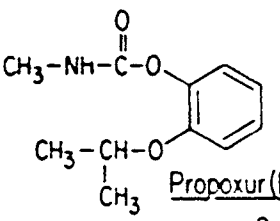
Table CXXI

Structural Formulas of Carbamate Pesticides

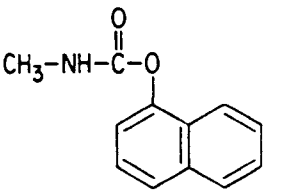
Tested for Carcinogenicity



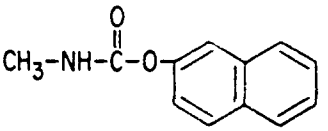
Zectran (Mexacarbate)



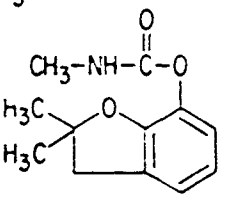
Propoxur (Baygon)



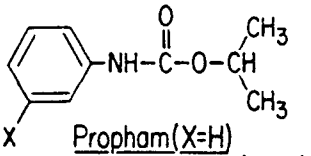
Carbaryl (α-Sevin)



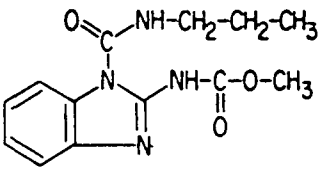
β-Sevin



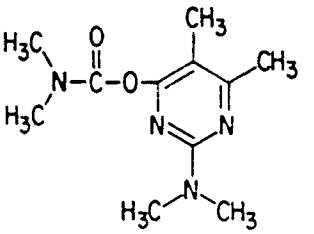
Carbofuran (Furadan)



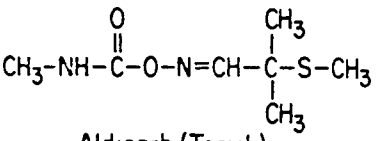
Propham (X=H)
Chloropropham (X=Cl)



Benomyl (Benlate)



Primicarb



Aldicarb (Temik)

Table CXXII

Carcinogenicity of Carbamate Pesticides

Compound	Species and strain[a]	Carcinogenicity (route)	Reference
Zectran [Mexacarbate, 4-(dimethylamino)-3, 5-dimethylphenyl-N-methylcarbamate]	Mouse, $B6C3F_1$	Lung, liver (oral)	(18, 19)
		None (s.c.)	(18)
		Liver, skin (oral) (marginal)	(149)
	Mouse, $B6AKF_1$	None (oral or s.c.)	(18, 19)
	Rat, Osborne-Mendel	None (oral)	(149)
Propoxur [Baygon, 2-isopropoxyphenyl N-methylcarbamate]	Rat, unspecified	None (oral)	(reported in ref. 83)
Carbaryl [Sevin, 1-naphthyl N-methylcarbamate]	Mouse, $B6C3F_1$ or B6AKF	None (oral or s.c.)	(18, 19)
	Mouse, A/Jax or C3H	None (s.c.)	(86)
	Mouse, ICR/Ha or A/J	None (oral)	(150)
	Mouse, A/He	No significant effect (i.p.)	(138)
	Rat, CF-N	None (oral)	(86)
	Rat, random bred	Connective tissue (oral)	(151)
		No significant effect (s.c.)	(151)

β-Sevin [2-Naphthyl N-methylcarbamate]	Mouse, CC57W	Liver, lung (oral)	(152)
		Lung (s.c.)	(152)
	Rat, random bred	Mammary gland (oral)	(152)
		Local sarcoma (s.c.)	(152)
Carbofuran [Furadan, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate]	Rat, unspecified	None (oral)	(26)
	Dog, unspecified	None (oral)	(26)
Propham [Isopropyl N-phenylcarbamate, IPC, Isopropyl carbanilate]	Mouse, strain C	None (oral, s.c., i.m., or intrapleural)	(153)
	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c.)	(18, 19)
	Mouse, A/He	None (i.p.)	(138)
	Rat, Osborne-Mendel	None (oral)	(153)
		Uterus (i.m.) (questionable)	(153)
	Rat, unspecified	None (oral)	(154)
	Hamster, Syrian golden	None (oral)	(155)

Chloropropham [Isopropyl N-(3-chlorophenyl)-carbamate, CIPC, isopropyl m-chlorocarbanilate]	Mouse, $B6C3F_1$ or $B6AKF_1$ or Swiss	None (oral or s.c.)	(18, 19, 155)
	Rat, albino	None (oral)	(156)
	Hamster, Syrian golden	None (oral)	(155)
Benomyl [Benlate, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate]	Rat, unspecified	None (oral)	(reported in ref 157)
	Dog, unspecified	None (oral)	(reported in ref 157)
Primicarb [2-Dimethylamino-5,6-dimethyl-pyrimidine-4-yl dimethylcarbamate]	Rat, unspecified	None (oral)	(reported in ref 83)
	Dog, unspecified	None (oral)	(reported in ref 83)
Aldicarb [Temik, 2-methyl-2-(methylthio)-propionaldehyde O-(methylcarbamoyl)-oxime]	Mouse, $B6C3F_1$	None (oral)	(159)
	Rat, unspecified	None (oral)	(Weil and Carpenter cited in ref. 158)
	Rat, Greenacre Lab	None (oral)	(Weil and Carpenter cited in ref. 158)
	Rat, F344	None (oral)	(159)
	Dog, unspecified	None (oral)	(Weil and Carpenter cited in ref. 158)

[a] $B6CF_1$ = (C57BL x C3H)F_1, $B6AKF_1$ = (C57BL x AKR)F_1

similarly treated $B6AKF_1$ mice were observed. A single s.c. injection of 10 mg/kg of the compound was without any effect in both strains (18, 19) The carcinogenicity of Zectran has recently been re-evaluated in the NCI bioassay program (149) Osborne-Mendel rats and $B6C3F_1$ mice were fed diets containing two dose levels of the compounds (time-weighted average 209 or 418 ppm for male rats, 339 or 678 ppm for female rats, 327 or 654 ppm for male mice, 68 or 135 ppm for female mice), no significant increase in tumor incidence occurred in dosed rats and female mice. Among male mice surviving beyond 56 weeks, significant association between Zectran treatment and the induction of hepatocellular carcinoma, s.c fibrosarcomas and skin fibromas was established by one statistical test (Cochran-Armitage) but not by another (Fisher) Thus, although there is some indication of potential carcinogenicity, there is no convincing evidence

Propoxur (Baygon) is a phenyl N-methylcarbamate with an isopropoxy group in the ortho position. In a two-year feeding study, a dietary level of 250 ppm was reported to have no ill effects in male and female rats. At 750 ppm, the liver weight of female rats was increased, although there were no other obvious adverse effects (reported in ref. 83), no details of the study are available

Carbaryl (Sevin, 1-Naphthyl N-methylcarbamate) is probably the most extensively studied carbamate pesticide. In a variety of mouse strains (including $B6C3F_1$, $B6AKF_1$, A/Jax, A/J, A/He, C3H, and ICR/Ha), no significant carcinogenicity could be demonstrated by various routes of administration (oral, i p., s c.)(18, 19, 86, 138, 150) Similarly, Carbaryl failed to induce tumors in

CF-N rats after feeding of diets containing up to 0 4% of the compound (86). The only evidence of potential carcinogenicity was provided by Andrianova and Alekseev (151) who showed that 4/10 rats receiving 30 mg/kg of the compound for 22 months developed tumors (3 fibrosarcoma, 1 osteosarcoma) Only 1/46 control rats developed a fibrosarcoma. When tested by s c. administration, the carcinogenicity of Carbaryl was not significant (151).

In contrast to the general lack of carcinogenicity of Carbaryl, β-Sevin (2-naphthyl N-methylcarbamate) was shown to be unequivocally carcinogenic in rodents (152) Daily oral administration of 10 mg β-Sevin to CC57W mice or 25 mg to random-bred white rats for 2-2½ years led to significant increases in the incidence of liver and lung tumors in mice and mammary tumors in rats (152) It is interesting to note that the introduction of a functional group (e.g., amine, mustard) into the β-position of naphthalene almost always confers carcinogenicity to the molecule Thus, β-naphthylamine (Section 5 1 2.2.1) is a well-known human bladder carcinogen. β-Naphthylamine nitrogen mustard (Section 5 2 1 1 1) induces lung tumors and local sarcomas in animals and is suspected to be a human carcinogen. It is therefore not surprising that β-Sevin is also carcinogenic.

Carbofuran (Furadan) is a relatively new carbamate pesticide containing a substituted benzofuranyl ring. In an industry-sponsored two-year feeding study, dietary levels of 25 or 20 ppm Carbofuran were reported to have no adverse effects in rats and dogs, respectively (26) The details of the study are not available The doses administered are probably well below the maximum tolerated levels.

Two alkyl N-phenylcarbamates, Propham and Chloropropham, that have been extensively used as herbicides, have been tested for carcinogenicity Both compounds have been consistently found to have no significant carcinogenic effect in various strains of mice, rats, and in Syrian golden hamsters (18, 19, 86, 153-156). The only possible evidence of carcinogenicity of Propham was provided by Hueper (153) who showed that 4/10 rats that survived 6 monthly intramuscular injections of 400 mg/kg Propham developed tumors (2 uterine adenomyomas, 1 adenofibroma of the groin, 1 mammary adenocarcinoma). One mammary adenoma was detected among 10 control rats The evidence may be of questionable significance because of the small number of animals used

Benomyl is a fungicide with a substituted benzimidazole ring linked to the amino group of methylcarbamate. It was reported to have very low toxicity in chronic studies In two-year feeding studies, dietary levels of 2500 ppm and 500 ppm were reported to have no adverse effect in rats and dogs, respectively (reported in ref. 157). The details of the study are not available.

Primicarb, a selective aphicide, is a N, N-dimethylcarbamate with a substituted heterocyclic aromatic ring. In two-year feeding studies, the "no effect" levels were reported to be 1 8 mg/kg for dogs and 250 ppm in the diet for rats (equivalent to 12 5 mg/kg) (reported in ref 83), no details are available

Aldicarb (Temik) is the first oxime carbamate pesticide registered It has been used as a substitute pesticide for some cancelled and suspended uses of DDT. The carcinogenicity of Aldicarb was first tested by Weil and Carpenter. Their unpublished data (reviewed by USEPA, ref 158) inducated that Aldicarb,

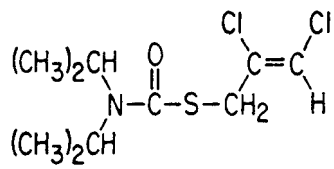
at dietary levels equivalent to daily intake of 0.1 mg/kg, did not cause any significant increase in the tumor incidences in an unspecified strain of rats ter two years This study was repeated by Weil (reviewed by USEPA, ref. 158) using Greenacre Laboratory Controlled Flora rats with essentially the same results. The sulfoxidized metabolites (sulfoxide and sulfone) of Aldicarb were also not carcinogenic. The "no adverse effect level" was estimated to be 0.3 mg/kg/day for the rat. The carcinogenicity of Aldicarb was also studied in beagle dogs Four groups of 6 dogs were fed diets containing 0, 0.83, 1.67 and 3.33 ppm Aldicarb (equivalent to daily intake of 0, 0.025, 0 05 and 0 1 mg/kg, respectively), no statistically measurable deleterious effects were observed in any of these groups (158) In a recent NCI bioassay (159), Aldicarb was fed to $B6C3F_1$ mice and F344 rats at two dose levels (2 or 6 ppm) for 103 weeks, no tumors occurred in either the rat or the mouse at incidences that could be related to the administration of the pesticide It was pointed out, however, that the doses used may not represent the maximal tolerated doses (159).

5.2.1.6.3.6 Thiocarbamate Pesticides. Thiocarbamate pesticides may be classified, on the basis of difference in chemical properties, into four groups S-chloroallyl thiocarbamate, dialkyldithiocarbamate, thiocarbamyl disulfide (or tetraalkylthiuram disulfide), and ethylenebisdithiocarbamate. Twenty such compounds have been tested for carcinogenicity The structural formulas of these compounds are depicted in Tables CXXIII and CXXV and the carcinogenicity data are summarized in Tables CXXIV through CXXVII.

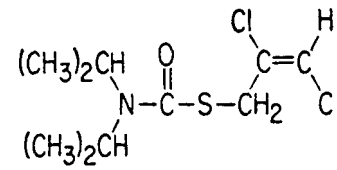
← Tables CXXIII & CXXIV

Table CXXIII

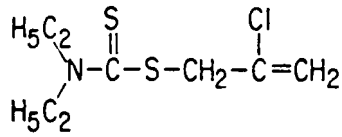
Structural Formulas of Thiocarbamate Pesticides Tested for Carcinogenicity



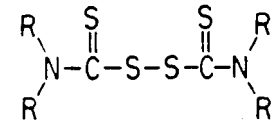
Diallate (cis-isomer)



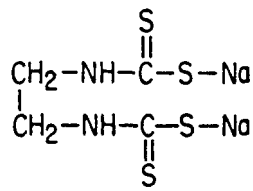
Diallate (trans-isomer)



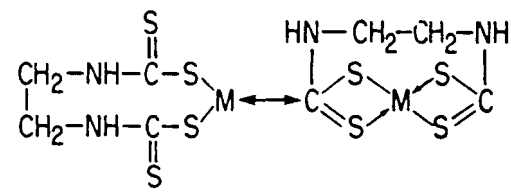
Sulfallate (CDEC)



Thiram (R=CH_3)
Disulfiram (R=C_2H_5)



Nabam



Maneb (M=Mn)
Zineb (M=Zn)

Table CXXIV

Carcinogenicity of S-Chloroallyl Thiocarbamates

Compound	Species and strain[a]	Principal carcinogenic effect (route)	Reference
Diallate [Avadex, S-(2, 3-dichloroallyl) N, N-diisopropyl thiocarbamate]	Mouse, $B6C3F_1$	Liver, lung (oral)	(18, 19)
		Hematopoietic system (s c.)	(18)
	Mouse, $B6AKF_1$	Liver (oral)	(18, 19)
		None (s c.)	(18)
	Rat, Charles River CD	Mammary gland, various sites (oral)	(160, 161)
Sulfallate [2-Chloroallyl N, N-diethyl dithiocarbamate]	Mouse, $B6C3F_1$	Lung, mammary gland (oral)	(162)
	Rat, Osborne-Mendel	Forestomach, mammary gland (oral)	(162)

[a] $B6C3F_1$ = (C57BL X C3H)F_1, $B6AKF_1$ = (C57BL X AKR)F_1

S-Chloroallyl thiocarbamates have recently attracted much attention because of their carcinogenicity (Table CXXIV) and/or mutagenicity (Table CXV). S-Chloroallyl thiocarbamates have been used as selective pre-emergence herbicides. In a preliminary NCI bioassay, daily oral administration of 215 mg/kg body weight of Diallate (Avadex) was found to cause significant increase in the incidences of liver and lung tumors in $B6C3F_1$ mice and liver tumors in $B6AKF_1$ mice (18, 19). A single s.c dose (1 g/kg) induced reticulum-cell sarcoma in $B6C3F_1$ mice (18). Ulland et al. (160) reported in a 1973 abstract that Diallate was carcinogenic in Charles River CD rats after feeding for two years The details of the study were provided to the Carcinogen Assessment Group of the U.S. Environmental Protection Agency (161), which concluded that the compound was significantly carcinogenic in male rats at high dose and in female rats at low dose Re-interpretation by this government agency (161) of carcinogenicity data supplied by an industrial laboratory also indicated that Diallate was carcinogenic, inducing a statistically significant excess of mammary gland carcinoma in female rats These data, along with the results of mutagenicity and neurotoxicity studies, led the Environmental Protection Agency (161) to propose the banning of the use of the pesticide.

Sulfallate is closely related to Diallate. Sulfallate has been tested for carcinogenicity in a recent NCI bioassay study (162). Osborne-Mendel rats and $B6C3F_1$ mice were fed diets containing two dose levels of the compound (the time-weighed averages are 250 and 404-410 ppm for rats and 908-949 and 1815-1897 ppm for mice) for 78 weeks The results of the study indicated that

Sulfallate was carcinogenic in both rodent species. It induces mammary gland tumors in the females of both species, tumors of the forestomach in male rats, and tumors of the lung in male mice (162) Triallate is another member of the S-chloroallyl thiocarbamate class, it is an analog of Diallate, with an additional chlorine atom on the terminal carbon of the allyl group. The carcinogenicity of the compound has not been tested. However, mutagenicity studies of the compound revealed that it behaved in a manner closely similar to Diallate and Sulfallate (see Section 5.2.1 6 2 2). Based on structural analogy and mutagenicity data, the evidence is compelling to suggest that Triallate would also be carcinogenic.

Dialkyldithiocarbamates have been used in industry as accelerators of rubber processing and in agriculture as herbicide. Thirteen dialkyldithiocarbamates were included in the preliminary NCI carcinogenesis bioassay (18, 19) Some of these compounds have also been investigated by other investigators and re-evaluated in the recent NCI bioassay The results of these studies are summarized in Table CXXV. In the preliminary NCI bioassay (18, 19) none of the 13 compounds tested by a single s c injection was carcinogenic. By oral administration, however, 5 of the 13 compounds were either weakly or marginally active as carcinogens. These five included (a) sodium diethyldithiocarbamate which had marginal activity in inducing hepatomas (in) $B6C3F_1$ mice and lung tumors in $B6AKF_1$ mice, (b) potassium bis(2-hydroxyethyl) dithiocarbamate which induced hepatomas in both strains, (c) Ledate which had a marginal effect in inducing reticulum-cell sarcomas (in) $B6C3F_1$ mice, (d) ethyl selenac which induced hepatomas (in) $B6C3F_1$ mice and (e) ethyl tellurac which induced

lung tumors (and) hepatomas in $B6AKF_1$ mice. There is no simple structure-activity relationship associating chemical structure with carcinogenicity It is possible, however, that the metal ion may play some role in the carcinogenicity

The carcinogenicity of sodium diethyldithiocarbamate, Ledate and ethyl tellurac has been re-evaluated in NCI bioassays (163-165) in F344 rats and $B6C3F_1$ mice. At maximally tolerated doses, none of these compounds were unequivocally carcinogenic. Only ethyl tellurac induced a dose-related increase in mesothelioma in male rats but the increase was not significantly different from that of the controls In male mice, the incidence of ethyl tellurac-induced adenoma of the Harderian gland (lacrimal gland of the eye) was significantly higher, however, a dose-related trend could not be established (165).

Potassium bis(2-hydroxyethyl)dithiocarbamate is the only compound shown to be carcinogenic (in Charles River CD rats) by oral administration (160), the details of the study were not given

The lack of carcinogenicity of Ziram was shown by Chernov and Khitsenko (166) in C57 and strain A mice Rochester strain rats receiving Ziram did not have a higher incidence of pituitary and thyroid gland tumors than untreated animals of the same strain (167) In random-bred rats Ziram was considered carcinogenic (151). Given orally twice weekly for 22 months, Ziram (70 mg/kg) induced tumors of the liver and connective tissue in 4/10 surviving rats. By s. c. administration, 3/10 rats developed tumors in the liver, colon and subcutaneous tissue Only 1/46 control rats developed a tumor. The survival rate of the dosed animals was very low

Two thiocarbamyldisulfides (Thiram and Disulfiram) have been tested for carcinogenicity (Table CXXVI). Thiram was found to be noncarcinogenic in the preliminary NCI bioassay (18, 19). Disulfiram was, however, marginally active, by oral administration to $B6C3F_1$ mice it induced hepatomas and lung tumors, and by s c. administration to $B6AKF_1$ mice it induced reticulum--cell sarcomas in the females (18). These marginal activities of Disulfiram could not be confirmed in the more thorough recent NCI bioassays (168); oral administration of maximally tolerated doses of Disulfiram to F344 rats (300 or 600 ppm) and $B6C3F_1$ mice (500 or 2000 ppm for males, 100 or 500 ppm for females) did not bring about any significant increase in tumor incidence The lack of carcinogenicity of Disulfiram in Sprague-Dawley rats was also reported by Schmähl et al. (169)

Table CXXVI

Ethylenebisdithiocarbamates have been widely used as fungicides The carcinogenicity of three such compounds (Nabam, Maneb and Zineb) has been tested (Table CXXVII). In a preliminary NCI bioassay, none of these three compounds was found to be carcinogenic by oral administration to $B6C3F_1$ and $B6AKF_1$ mice (18, 19). Only Zineb, by s. c. administration, caused a slight increase in the incidence of reticulum-cell sarcomas in male $B6C3F_1$ mice (18). Balin (170) administered orally 6 weekly doses of 500 mg/kg Maneb to C57BL and strain A mice Increases in the incidences of lung tumors in both strains were observed, however, the increase was statistically significant only in strain A mice (170). Andrianova and Alekseev (151) administered Maneb orally (335 mg/kg twice weekly for 22 months) and subcutaneously (12 5 mg) to random

Table CXXVII

Table CXXVI

Carcinogenicity of Thiocarbamyl Disulfides

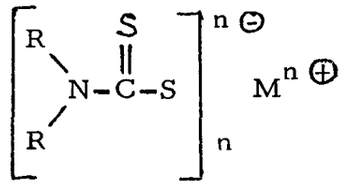
Compound	Species and strain[a]	Principal carcinogenicity target (route)	Reference
Thiram [Tetramethylthiuram disulfide, TMTD, bis(dimethylaminothiocarbamyl) disulfide]	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c)	(18, 19)
Disulfiram [Tetraethylthiuram disulfide, bis(diethylaminothiocarbamyl) disulfide]	Mouse, $B6C3F_1$	Liver, lung (oral) (marginal)	(18, 19)
		None (s c)	(18)
		None (oral)	(168)
	Mouse, $B6AKF_1$	None (oral)	(18, 19)
		Hematopoietic system (s c.) (marginal)	(18)
	Rat, Sprague-Dawley	None (oral)	(169)
	Rat, F344	None (oral)	(168)

[a] $B6C3F_1$ = (C57BL X C3H)F_1, $B6AKF_1$ = (C57BL X AKR)F_1

Table CXXV

Carcinogenicity of Metallic Dithiocarbamates

Structure



Compound	M	n	R	Species and strain[a]	Principal organs affected (route)	Reference
Sodium diethyldithiocarbamate	Na	1	C_2H_5-	Mouse, $B6C3F_1$	Liver (oral) (marginal)	(18, 19)
					None (s. c.)	(18)
					None (oral)	(165)
				Mouse, $B6AKF_1$	Lung (oral) (marginal)	(18, 19)
					None (s. c.)	(18)
				Rat, F344	None (oral)	(165)
Potassium bis-(2-Hydroxyethyl) dithiocarbamate	K	1	$HOCH_2CH_2$-	Mouse, $B6C3F_1$ or $B6AKF_1$	Liver (oral)	(18, 19)
					None (s. c.)	(18)
				Rat, Charles River CD	Various sites (oral)	(160)
Ziram [Zinc dimethyldithiocarbamate]	Zn	2	CH_3-	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s. c.)	(18, 19)
				Mouse, C57 or A	No significant effect (oral)	(166)
				Rat, Rochester	Pituitary, thyroid (oral) (not considered significant)	(167)
				Rat, random bred	Liver, connective tissue (oral)	(151)
					Various sites (s. c.)	(151)

Ethyl zimate [Zinc diethyldithiocarbamate]	Zn	2	C_2H_5-	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c)	(18, 19)
Butyl zimate [Zinc dibutyl dithiocarbamate]	Zn	2	C_4H_9-	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c)	(18, 19)
Cumate [Cupric dimethyldithiocarbamate]	Cu	2	CH_3-	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c)	(18, 19)
Ethyl cadmate [Cadmium diethyldithiocarbamate]	Cd	2	C_2H_5-	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c)	(18, 19)
Ledate [Lead dimethyldithiocarbamate]	Pb	2	CH_3-	Mouse, $B6C3F_1$	Hematopoietic system (oral) (marginal)	(18, 19)
					None (oral)	(163)
					None (s c)	(18)
				Mouse, $B6AKF_1$	None (oral or s.c.)	(18, 19)
				Rat, F344	None (oral)	(163)

Bismate [Bismuth dimethyldithiocarbamate]	Bi	3	CH_3-	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c.)	(18, 19)
Ferbam [Ferric dimethyldithiocarbamate]	Fe	3	CH_3-	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c.)	(18, 19)
				Rat, Rochester	None (oral)	(94, 167)
Methyl selenac [Selenium dimethyldithiocarbamate]	Se	4	CH_3-	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s.c.)	(18, 19)
Ethyl selenac [Selenium diethyldithiocarbamate]	Se	4	C_2H_5-	Mouse, $B6C3F_1$	Liver (oral)	(18, 19)
					None (s.c.)	(18)
				Mouse, $B6AKF_1$	None (oral or s.c.)	(18, 19)
Ethyl tellurac [Tellurium diethyldithiocarbamate]	Te	4	C_2H_5-	Mouse, $B6C3F_1$	None (oral or s.c.)	(18, 19)
					Harderian gland (oral)	(164)
				Mouse, $B6AKF_1$	Lung, liver (oral)	(18, 19)
					None (s.c.)	(18)
				Rat, F344	None (oral)	(164)

[a] $B6C3F_1$ = (C57BL X C3H)F_1, $B6AKF_1$ = (C57BL X AKR)F_1

Table CXXVII

Carcinogenicity of Metallic Ethylenebisdithiocarbamates

Compound	Species and strain[a]	Principal carcinogenicity target (route)	Reference
Nabam [Disodium ethylenebisdithiocarbamate]	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s. c.)	(18, 19)
Maneb [Manganese ethylenebisdithiocarbamate]	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s. c)	(18, 19)
	Mouse, C57Bl	No significant effect (oral)	(170)
	Mouse, strain A	Lung (oral)	(170)
	Rat, random bred	Various sites (oral or s. c.) (significance questionable)	(151)
Zineb [Zinc ethylenebisdithiocarbamate]	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral)	(18, 19)
	Mouse, $B6C3F_1$	Hematopoietic system (s. c.)	(18)
	Mouse, C57Bl	Lung (oral)	(166)
	Mouse, strain A	No significant effect (oral)	(166)
	Rat, unspecified	No significant effect (oral)	(97)
	Rat, random bred	No significant effect (oral)	(151)
	Rat, random bred	Various sites (s. c.) (significance questionable)	(151)

[a] $B6C3F_1 = (C57BL \times C3H)F_1$, $B6AKF_1 = (C57BL \times AKR)F_1$

bred rats, the induction of tumors in various sites (mammary gland, s c tissue, thyroid gland, connective tissue) was noted. The significance of this study may be questionable because of the low survival rate of dosed animals. The carcinogenicity of Zineb was investigated by Chernov and Khitsenko (166). Strain A and C57BL mice were given 6 weekly doses of Zineb (3.5 g/kg) and killed 3 months later, 6 of the 79 C57 mice developed lung tumors compared to 0/87 control In strain A mice, the incidence of lung tumors in the Zineb-treated group (35%) was not significantly different from that of the control group (31%) Blackwell-Smith et al (97) fed groups of 10 rats of each sex diets containing 0, 500, 1,000, 2,500, 5,000 or 10,000 ppm of Zineb There was no significant carcinogenic effect associated with the administration of the chemical. In the most affected group (1,000 ppm) only 4/20 rats developed tumors, for comparison, 2/20 control rats also developed tumors The study by Andrianova and Alekseev (151) indicated that Zineb (285 mg/kg, twice weekly for 22 months) was not significantly carcinogenic in random-bred rats after oral administration. By s c. administration (20 mg/kg), however, 4 of the 6 surviving rats developed tumors (1 hepatoma, 1 fibrosarcoma, 1 spindle cell sarcoma, 1 subcutaneous rhabdomyosarcoma) The significance of the latter study is questionable, however, because of the low survival rate and the small number of animals involved.

5.2.1.6.3.7 Substituted Urea Compounds Substituted urea compounds are closely related in structure to carbamates Like carbamates and thiocarbamates, substituted urea compounds have been used as pesticides Similarly

to urethan, some substituted urea compounds (e g , Carbromal) have sedative and hypnotic activity Only a few substituted ureas have been tested for carcinogenicity. The structural formulas are shown in Table CXXVIII and the carcinogenicity data summarized in Table CXXIX. ← Tables CXXVIII & CXXIX

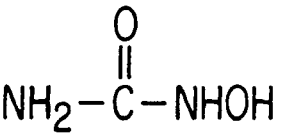
Hydroxyurea was found noncarcinogenic in two strains of mice Muranyi-Kovacs and Rudali (171) administered intraperitoneally to 50 XVII/G mice doses of 1, 3, 5 mg hydroxyurea at the age of 2, 8, 15 days and then 10 mg/week from day 30 to 1 year No significant carcinogenic effects were observed, in fact, the lung tumor incidence in the hydroxyurea-treated group (45. 7%) was lower than that of untreated animals (60%) which had a high spontaneous tumor incidence. In the experiments of Bhide and Sirsat (172) Swiss mice were treated subcutaneously with hydroxyurea, only one male developed lung tumors and only one female developed a mammary fibrosarcoma (an incidence not different from that of the controls).

Cabromal is a mild central nervous system depressant, similar in biological action to urethan It was selected by the NCI (173) for carcinogenicity testing because of its similarity to urethan. Fisher 344 rats and $B6C3F_1$ mice were given dietary levels of 1, 250 or 2, 500 ppm Carbromal for 103 weeks and 78 weeks, respectively, and were observed up to 105 weeks No significant positive association between administration of the compound and the level of tumor incidence was noted There was some indication of dose-related incidence of adrenal pheochromocytomas (in) male rats, but the effect was not statistically significant.

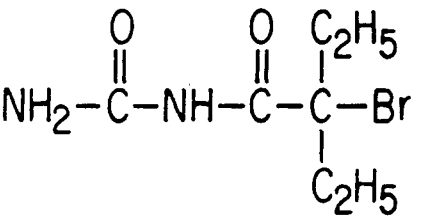
Table CXXVIII

Structural Formulas of Substituted Urea Compounds

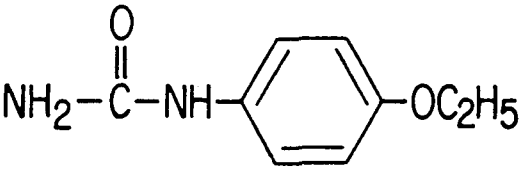
Tested for Carcinogenicity



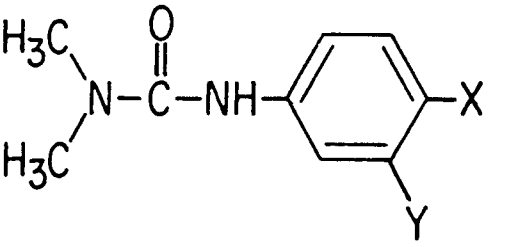
Hydroxyurea



Carbromal



Dulcin



Monuron (X=Cl, Y=H)
Diuron (X=Y=Cl)

Table CXXIX

Carcinogenicity of Substituted Urea Compounds

Compound	Species and strain[a]	Principal carcinogenicity target (route)	Reference
Hydroxyurea	Mouse, XVII/G	None (i p)	(171)
	Mouse, Swiss	No significant effect (s c)	(172)
Carbromal [N-(Aminocarbonyl)-2-bromo-2-ethyl butanamide, (2-bromo-2-ethyl-butyryl) urea]	Mouse, $B6C3F_1$	None (oral)	(173)
	Rat, F344	None (oral)	(173)
Monuron [N'-(4-Chlorophenyl) N, N-dimethyl urea]	Mouse, $B6C3F_1$	None (oral or s c)	(18, 19)
	Mouse, $B6AKF_1$	Lung (oral)	(18, 19)
		None (s. c.)	(18)
	Mouse, random bred or C57Bl	Liver, lung (oral)	(174)
	Rat, random bred	Liver, lung (oral)	(174)
Diuron [N'-(3, 4-Dichlorophenyl) N, N-dimethyl urea]	Mouse, $B6C3F_1$ or $B6AKF_1$	None (oral or s c.)	(18, 19)

[a] $B6C3F_1 = (C57BL \times C3H)F_1$, $B6AKF_1 = (C57BL \times AKR)F_1$

Dulcin (4-ethoxyphenyl urea), originally produced as an artificial sweetener, may also be regarded as 4-ethoxy-N-carbamylaniline. Classified as a substituted monocyclic aromatic amine, Dulcin has been discussed in Section 5.1.2.1- in Vol IIB. Its use as an artificial sweetener has now been banned in most countries.

Phenylalkylureas are rapidly gaining importance as herbicides. Two such compounds (Monuron and Diuron) have been tested for carcinogenicity. In the preliminary NCI bioassay, Monuron was found to be inactive in $B6C3F_1$ mice but induced lung tumors after oral administration to $B6AKF_1$ mice (18, 19). A more convincing demonstration of carcinogenicity of Monuron was subsequently provided by Rubenchik et al (174). One hundred random-bred rats were fed diets containing 450 mg/kg of Monuron for 18 months. Fifty random-bred mice and 45 C57Bl mice received 6 mg Monuron in milk once a week for 15 weeks. Significant increases in tumor incidence were observed in all treated rodents, the liver and the lung were the most affected organs. The tumor incidence was 46.5% in treated rats (compared to 0% in controls), 56.5% in random-bred mice (control figure not available), and 26.9% in C57Bl mice (control 3.9%). Diuron, the only other phenylalkylurea studied, was inactive in the preliminary NCI bioassay (18, 19). However, in view of the preliminary nature of the latter study and the previous demonstration of the carcinogenicity of Monuron, further studies of the compound should be undertaken.

5.2.1.6.3.8 Transplacental and Lactationally Mediated Carcinogenesis. The transplacental carcinogenicity of urethan in the mouse has been extensively

studied. The demonstration by Nomura et al (62) that radioactively labeled urethan may readily cross the placental barrier throughout the gestation period, irrespectively of the presence of unlabeled compound, indicates the easy accessibility of the carcinogen to the fetus. Larsen (175) was the first to demonstate the transplacental carcinogenicity of urethan Pregnant strain A mice, given a single i.p. or i.v. injection of 25 mg urethan 1-5 days before term, gave birth to offspring with increased incidence of lung tumors When sacrificed 6 months after birth, the incidence was 100% and the multiplicity was 8.9-10 tumors/mouse among offspring whose mothers received urethan one day before parturition. For offspring whose mothers received urethan 2-5 days before term, the incidence was 60-80% and the multiplicity was substantially lower (with an average of 1-2 tumors/mouse). Similar findings have been reported by Smith and Rous (176) using strain C mice, by Klein (177) using AxC mice, and by DiPaolo (178) and Kolesnichenko (179) using strain A mice Veselinovitch et al (180) injected urethan (0 5 g/kg) subcutaneously for 5 consecutive days to Swiss or C3H mice starting on day 7 or day 11 of gestation, respectively. The treatment resulted in increased incidence of hepatomas in the C3H offspring and of ovarian tumors in the offspring of both strains. Bojan (181) treated pregnant CFLP mice with a single dose (1 g/kg) of urethan on day 10, 11, 12 or 19 of gestation, the tumor incidences observed among offspring sacrificed 35 days after birth were 0%, 0%, 3-4%, and 31-42%, respectively Anderson (182) has stressed that for some strains, the offspring must be allowed to live for a sufficiently long duration in order to detect transplacental carcinogenicity In BALB/c mice, for example, a high incidence of lung tumors was observed only if they were sacrificed 8-12 months after birth

The chronological relationship between organogenesis and transplacental carcinogenesis has been investigated by Nomura and associates (61, 183). Pregnant ICR-JCL mice were given a single s c. dose (1 g/kg) of urethan on day 5, 7, 9, 11, 13, 15, 17 or 19 of gestation. The incidence of lung tumors in the offspring was not significantly different between control and the groups treated before day 11. The incidence then rose to 30.8%, 64.3% and 71.4% for the groups treated on day 13, 15 and 17, respectively. Embryological studies revealed that this sensitive period of carcinogenesis coincides with the period when the lung buds appear (on day 12) and grow actively. Of the group treated on day 19, 64.9% of the offspring developed lung tumors Within this group, however, there was a large difference in the incidence and multiplicity between the offspring that were born within 24 hr of urethan treatment to mothers (100%, average 4 56) and those born over 24 hours after treatment (31 6%, average 0 47) It was suggested (61) that, due to the long retention of urethan, a significant proportion of urethan could be transferred into the newborns, thus enhancing the carcinogenic effect of urethan. In addition to lung tumors, hepatomas were found in male but not in female offspring. The incidences were 6.9, 15 and 7 1% if the mothers were treated on day 11, 13 or 15 of gestation, respectively Microscopic examination of the embryos indicated that liver "buds" first appeared on day 10.

The modifying effect of a number of exogenous and endogenous factors on the transplacental carcinogenicity of urethan has been explored. DiPaolo (178) showed that exposure of pregnant mice during urethan treatment (given

within 24 hours before parturition) to an atmosphere containing either 100% oxygen (hyperoxia) or 10% oxygen (hypoxia) led to the induction of greater number of lung tumors than in those mice kept in normal room air. Hyperoxia or hypoxia alone did not significantly affect the lung tumor yield Apparently, a change in oxygen concentration may alter the susceptibility of lung cells to urethan The role of thymus has been studied using genetically athymic mice (BALB/c nu/nu, nu being gene for nude, hairlessness and athymia). Female nu/+ mice, mated with nu/+ males, were given urethan on day 17 or 19 of gestation and the incidences of primary lung tumors in the nude (nu/nu) and phenotypically normal (nu/+ or +/+) mice were compared (184). There was no significant difference. Histologically, however, the tumors in nude mice appeared to be more invasive and atypical, suggesting that the absence of thymus may increase the metastatic tendency of primary tumors An interesting diaplacental initiation-promotion experiment has been reported by Goerttler and Lohrke (124). NMRI mice were treated prenatally with urethan (3 daily doses of 60 mg/kg between gestation day 14-21) and postnatally with an active promotor, 12-O-tetradecanoylphorbol-13-acetate (TPA) A broad spectrum of tumors at the site painted with TPA and in other tissues have been observed. In general, the tumor incidences with the combined treatment exceeded those due to spontaneous incidence and those produced by urethan alone The skin carcinomas developed only after combined treatment of urethan and TPA. The results indicate the occurrence of transplacental tumor-initiation Furthermore, the relatively low doses required to initiate the tumor emphasize the high susceptibility

of the fetus. The transplacental carcinogenicity of urethan may also be enhanced by further exposing the suckling offspring to urethan via mother's milk (see also discussion below), and these carcinogenic effects appear to be additive (61, 183).

The transplacental carcinogenicity of urethan has also been tested in MRC rats. A low incidence (4 5%) of hepatomas and tumors of the heart were noted in offspring whose mothers received a single dose of urethan four days before term.

In contrast to the extensive study of urethan, the transplacental carcinogenicity of other carbamates and related compounds has been virtually unexplored. Zineb is probably the only dithiocarbamate that has been tested and reported Kvitnitskaya and Kolesnichenko (185) injected a single dose of 8 mg Zineb to 18 strain A mice during the second half of pregnancy. Eleven of these 18 produced 38 offspring, of which only 20 survived. After 4 months, six of these offspring were found to have lung adenomas, no such tumors were detected in control mice of similar age.

The maternal transfer of urethan is not limited to the transplacental route Several investigators have reported increased tumor incidence in suckling mice whose mothers were treated with urethan during lactation, suggesting the transfer of urethan via mother's milk De Benedictis et al. (186) noted first that 25/32 (78%) Swiss mice developed lung tumors 210 days after receiving milk from mothers treated with urethan (30 mg, p.o., 1st, 3rd and 5th day after parturition) The possible contamination of the suckling mice from sources

other than maternal milk may be excluded, because suckling mice caged in the presence of untreated lactating female and urethan-treated adult male did not develop tumors to any significant extent Similar results were obtained by Nomura-(183) with ICR/JCL mice that were suckled by urethan-treated lactating mothers. The effect was greatest if the mothers were repeatedly treated shortly after delivery. All 10 suckling mice developed lung tumors (average 13.6/mouse) 32 weeks after receiving milk from mothers treated with 4 subcutaneous doses (1 g/kg) of urethan on the 2nd, 4th, 6th and 8th day after parturition Similar treatments on days 2, 7, 12, 17 or 14, 16, 18, 20 postpartum led to tumor incidences of 58% and 56%, respectively For controls, the incidence was only 2 4% Essentially the same finding was reported by Bojan (181) using CFLP mice Lactating mothers were treated with 4 doses (0.5 g/kg) of urethan on 4 consecutive days and allowed to nurse two groups of suckling mice aged 1-4 days or 12-16 days When autopsied 5 weeks after treatment, the former had a tumor incidence of 12-16% whereas the latter had a tumor incidence of only 4-5%

5 2.1 6.3 9 Modification of Carcinogenesis Induced by Carbamates and Related Compounds. As is the case with many other chemical carcinogens, the carcinogenicity of carbamates and related compounds may be modified by a variety of endogenous and exogenous factors Urethan (ethylcarbamate), in particular, has been extensively used as a model multipotential carcinogen in the study of various modifying factors. age, sex, host immune response, diet, physical trauma, exogenous chemicals, viral interaction, radiation, and other physical factors In addition to urethan, a few carbamate pesticides have been

investigated because of their potential risk to humans. While the major findings of these studies are briefly outlined in this sub-section, detailed discussion will be presented in a future volume of this series

Age. As previously mentioned in Section 5.2.1.6.3.2, newborn and preweanling animals have a greater susceptibility to the carcinogenic action of urethan. The greater susceptibility is particularly evident in the induction of pulmonary and hepatic tumors and leukemia. A variety of investigators have extensively studied the age effect. Some of the representative studies are described below

Rogers (187) was among the first to demonstrate the age effect in urethan-induced lung tumorigenesis. He administered a single i.p dose of urethan (1 g/kg) to Swiss mice at various ages and recorded the development of lung tumors 7 weeks later. Significantly higher susceptibility of the younger animals particularly during the first 6 weeks, was noted. The tumor incidences and multiplicity were, respectively, 100% and 6 1 tumors/mouse for 2-week-old, 92% and 5.4 for 4-week-old, 88% and 3.6 for 6-week-old, 84% and 2.6 for 8-week-old and 76% and 3 6 for 10-week-old mice The age effect was even more dramatic at lower urethan doses. At the dose of 0.25 g/kg, 16/19 (84%) mice developed tumors after treatment at the age of 3 weeks compared to 4/17 (23%) after treatment at the age of 8 weeks. Similar results have been observed with Swiss (186), A/Jax (116) and CFLP (181) mice. However, the age-dependence of pulmonary susceptibility to urethan is probably strain-specific, no significant age-difference has been observed in the development of urethan-induced lung tumors in some other strains of mice (188, 189)

Urethan rarely induces hepatic tumors in adult mice, however, when given to neonatal or infant animals, high incidences of hepatomas have been noted The role of age in urethan-induced hepatocarcinogenesis in Swiss mice has been investigated by Chieco-Bianchi et al. (190). Subcutaneous injection of urethan (1 g/kg) to newborn, 5-, 20- and 40-day-old mice led to the induction of liver tumors in 87, 70, 8 and 0% male and 9, 18, 0 and 0% female mice, respectively. In $B6AF_1/J$ hybrid mice, the most sensitive period was reported to be around the 7th day of age (188). Oral administration of 1 g/kg urethan to newborn, 7-, 14-, 21- and 28-day-old mice elicited hepatomas in 45, 91, 80, 57 and 17% male and 35, 77, 43, 5 and 5% female mice, respectively. There was no significant difference in the incidence of lung tumors (ranging from 84-100%) in this strain (188). Vesselinovitch et al. (189) administered i.p injections of urethan to $(C57 \times C3H)F_1$ mice starting at day 1, 4 or 175 of age. The incidences of hepatomas were 86%, 100%, 0-6 8% in males and 0%, 27%, 0% in females, respectively. In contrast, the lung and Harderian gland adenomas were observed with similar incidences in the groups receiving the same amount of urethan starting at day 4 or 175 of age.

The hematopoietic system also exhibits significant age-dependence in the susceptibility to the carcinogenic action of urethan Several investigators reported the lack of leukemogenic action of urethan in adult mice (e.g , ref. 191). Fiore-Donati et al. (192) studied the age effect with Swiss mice Mice given a single dose of urethan at the age of 1, 5 or 40 days developed leukemia with incidences of 21 6, 17.9 and 3.2%, respectively. Vesselinovitch and Mihailovich

(193) found that the most susceptible period is the very early neonatal stage. They administered to (C57 x C3H)F_1 mice total doses of 2 1, 3.0 or 4.2 g/kg starting at the age of 1 day or 7 days. The incidences of leukemia for the three doses in mice started on the first day of life were 7, 32 and 74%, whereas those of mice started on day 7 were 0, 7 and 38%, respectively.

In addition to the mouse, the age-dependence of susceptibility to urethane has been observed in the rat and hamster. Tannenbaum et al. (14) showed that urethan potentiates the formation of mammary tumors in Sprague-Dawley rats by increasing the multiplicity and reducing the latent period. Although their study was not designed to demonstrate age effect, it is evident from their data that the potentiating effect was greatest if the animals were treated starting at the first week of their life. The effect declined as the starting age of treatment increased. With Wistar-derived MRC rats, Kommineni et al (126) showed that the incidences of both neurilemmomas and hepatomas were significantly higher in rats treated neonatally than those treated from $4\frac{1}{2}$ or $6\frac{1}{2}$ weeks of age. In contrast, tumors of the thyroid gland were observed only in adult but not in neonatally treated rats. In the Syrian golden hamster, Toth (134) noted a substantially higher number of tumors of the intestines in animals treated neonatally than those treated starting at 8 weeks of age. On the other hand, an opposite effect was observed in the induction of tumors of the forestomach, whereas no age differences were found in the induction of skin tumors, thyroid, lung and other tumors.

Thus, age has a significant effect on the animal susceptibility to urethan. The effect is species-, strain- and target organ-specific In general, greater

susceptibility is associated with younger animals, this could be attributed to the slower rate of catabolism of urethan (194), higher proliferative activity of the immature cells and/or lower immune competence.

Sex. The sex of the animal plays an important role in the induction of tumors by urethan. Sex difference appears to be specific for certain organs or tissues, and is often species- or strain-dependent Probably the most consistent findings of sex difference are the greater susceptibility of male mice to hepatocarcinogenesis and female mice to leukemogenesis by urethan. In experiments with newborn or infant Swiss (190), C3Hf (195), B6AF$_1$/J (188, 196) and C57 X C3H hybrid (197, 198) mice, the incidences of urethan-induced hepatomas were invariably higher in males than in females. The greater susceptibility of the males has also been observed in young adult BALB/c (199) and "Hall" strain (200) mice receiving urethan after partial hepatectomy. In contrast to hepatocarcinogenesis, higher incidences or earlier development of neoplasms of the hematopoietic system have been found in female C3Hf (195) and dd (201, 202) mice after neonatal exposure to urethan.

The role of sex hormones in the modification of organ susceptibility to urethan has been studied by several investigators. Liebelt et al (195) administered a single dose of urethan to newborn C3Hf mice and allowed them to live out their lifespan During autopsy of the tumor-bearing animals, the type of sex hormone stimulation in the animals was assessed on the basis of gross and microscopic examination of the gonads, reproductive tract, submaxillary glands (a good indicator of androgenic stimulation) and kidneys There was a high

correlation between the occur ence of hepatomas and evidence of androgenic stimulation. All males that developed hepatomas had stimulated seminal vesicles and virtually all females bearing hepatomas had evidence of androgenic stimulation of the kidney and submaxillary glands. On the other hand, reticular tissue neoplasms were associated with estrogenic stimulation. There was no relationship between occurrence of lung tumors and specific type of sex hormone stimulation (195) In dd mice, the development of urethan-induced thymic lymphoma occurred much earlier and faster in females than in males. The testes were reported to exert an inhibitory influence on the thymus which could account for the later development of thymic lymphomas in male mice (201, 202). The effect of gonadectomy on the induction by urethan of hepatomas in 7-day-old (C57 X C3H)F_1 mice has been studied by Vesselinovitch and Mihailovich (197). In control (sham-operated) mice the incidences of hepatomas were 96% in males and 20% in females. The orchidectemized males had a significantly lower (62%), and the ovariectomized group a higher (67%), incidence and multiplicity of hepatomas than their corresponding non-castrated groups. Thus, gonadectomy practically abolished the sex difference in the induction of hepatomas by urethan

Immune response. Impairment of host immune competence is often associated with an enhancement of tumor development Urethan itself is an immunodepressant (albeit weak), as demonstrated by the impairment of allograft or homograft rejection response in urethan-treated mice (203-205). The immunodepressant activity is more pronounced in newborn than in adult mice

(203). It is not known whether the immunodepressant effect of urethan is a component factor of its carcinogenic activity.

The host immune system appears to play an important role in modifying the development of urethan-induced lung tumors. Trainin and associates have shown immunological impairment in Swiss mice as a result of neonatal thymectomy (206), thymectomy, and treatment with antilymphocyte serum (207) or autoimmune lymphocytes (208) invariably led to an increase in the incidence of urethan-induced lung tumors. Even a short duration of antilymphocyte serum treatment, permitting later immunologic recovery, was sufficient to enhance the host carcinogenic susceptibility if the carcinogen was administered during the period of immunodepression, suggesting that the early stage of urethan carcinogenesis was affected by immune manipulation (207). Innoculation of neonatally thymectomized mice with incompletely syngenic immunocompetent lymphoid cells, which further impaired the host immune competence via a "graft-versus-host" reaction, further enhanced the pulmonary susceptibility (209). On the other hand, repeated implantation of syngenic thymuses into neonatally thymectomized mice restored the immunocompetence and normalized the host response to urethan (207). The increased pulmonary susceptibility to urethan after neonatal thymectomy has also been shown by various other investigators using the same or different strains of mice (204, 210-212) In agreement with the above findings, Menard et al. (213) found that immunodepression by cortisone treatment enhances the pulmonary carcinogenicity of urethan in mice. On the other hand, pretreatment of mice with BCG (living bacillus

Calmette-Guerin) and trehalose-6,6-dimycolate ("cord factor") enhances the immunocompetence and reduces the number of urethan-induced lung tumors (214). Kraskovskii and Kagan (215) lowered the pulmonary carcinogenicity of urethan by innoculating the mice with immunocompetent embryo spleen (but not embryo liver) lymphoid cells.

In contrast to the above findings some negative correlations between immunodepression and enhancement of susceptibility have been reported Della Porta et al. (212) did not find any significant effect of splenectomy or cortisone treatment on the pulmonary carcinogenicity of urethan. Unlike thymectomized mice, genetically thymusless mice (BALB/c nu/nu) were not more susceptible to the pulmonary carcinogenic action of urethan after either prenatal (184) or postnatal exposure (216).

Diet. The modifying role of diet on the carcinogenicity of urethan has not been adequately explored, there are only a few and somewhat conflicting reports. Rogers (187) in 1951 reported that fasting of mice 19 hrs before urethan treatment had no significant effect on the incidence of lung tumors. On the other hand, Klarner and Klarner (217) showed that mice on a high-casein diet developed more lung tumors in response to urethan than those on a low-casein diet Newberne et al. (218) found that dietary choline deficiency (in spite of inducing liver cirrhosis) has a slightly inhibitory effect on the weak carcinogenic action of urethan in the rat. In a recent study by French (219), diets containing added nicotinamide, or choline, or myo-inositol significantly reduce the number of lung tumors induced by urethan in mice. The nicotinamide

effect appeared to be specific and could not be replaced by nicotinic acid. French (219) suggested that the carcinogenic effect of nicotinamide may, at least in part, be due to its ability to inhibit tumor-derived tRNA methylase. The possible mechanisms of action of choline and myo-inositol have not been explored.

Physical trauma Physical trauma is known to elicit unscheduled cell proliferation which in turn can promote chemical carcinogenesis. As discussed in Section 5.2.1.6.3.2, urethan has, in general, a weak or no carcinogenic action on the liver of adult animals, after partial hepatectomy, however, high incidences of liver tumors have been observed by various investigators. Hollander and Bentvelzen (220) reported that injection of 25 mg urethan into 2-month-old C3H/HeA male mice led to the induction of hepatomas in 11/36 (31%) animals. Partial hepatectomy one week after urethan treatment increased slightly the incidence to 50% (18/36) whereas when the partial hepatectomy preceeded urethan injection by 4 days, it strongly enhanced the incidence of hepatomas (81% of the animals developed one or more tumors). The incidence of lung tumors was not affected by partial hepatectomy. Similar results have been observed by Lane et al. (199) using BALB/c mice. Chernozemski and Warwick (221) using $B6AF_1$ mice, and Pound and Lawson (200) using Hall strain mice. In addition, significant sex difference was noted, the enhancing effect was substantially higher in male than in female mice (199, 200) Furthermore, the enhancing effect was positively correlated with the extent of partial hepatectomy and the mitotic activity of the liver thus produced (199, 200). Metabolic studies in intact and partially hepatectomized mice (200, 222) showed no

major difference, strongly supporting the concept that rapid cell proliferation and not the change in the metabolic rate of urethan accounts for the enhancement of susceptibility of liver to the carcinogenic action of urethan

Exogenous Chemicals. A variety of exogenous chemical agents are capable of modifying the carcinogenicity of urethan. On the other hand, a number of carbamates and related compounds modify the carcinogenicity of other chemicals. Some examples of such interactions are briefly outlined below.

The carcinogenic effects of combined treatment of urethan and a number of other chemical carcinogens have been investigated. Kawamoto et al (191) were the first to note that urethan potentiates the leukemogenic action of 3-methylcholanthrene in DBA mice This synergism, however, could not be demonstrated in the pulmonary carcinogenesis in strain A/J mice, instead, Yamamoto et al (223) found that the combined treatment with 3-methylcholanthrene and urethan (inducing an average of 27.4 nodules in all mice) was less carcinogenic than 3-methylcholanthrene alone (inducing 92 9 nodules in all mice). Bojan et al (224) have shown a clear syncarcinogenic effect between urethan and diethylstilbestrol in the induction of lymphoma in CFLP mice, the synergistic effect was the greatest if diethylstilbestrol was given 14 days after urethan treatment The finding is in accord with that of Kawamoto et al. (191) who reported that combined treatment of urethan and estradiol to castrated C57 mice induced leukemia in 59.4% of the mice compared to 6.6% for estradiol or 0% for urethan alone In a subsequent abstract presented by Yoshimura et al. (225),

urethan was reported to act synergistically with carcinogens present in engine exhaust gas. Female ICR-JCL mice exposed to the exhaust gas by inhalation and (to) urethan via drinking water, developed more malignant lung tumors than those exposed to exhaust gas or urethan alone. The combined effect of urethan and aflatoxin in the induction of liver tumors in the rat was tested by Newberne et al. (218). There was no evidence of synergism, in fact, slightly fewer liver tumors were observed after combined treatment than after aflatoxin alone.

The effect of combined treatment of urethan and some of its homologs has also been investigated. Thus, butyl and isoamyl carbamates have no significant effect on the pulmonary carcinogenic effect of urethan [redacted] (226). Pound (121) did not show any modifying effect of methyl-, n-propyl- and n-butyl-carbamates and several N-alkyl urethans on the induction of tumors in the skin, liver or lung by urethan in "Hall" strain mice. In contrast, Garcia and Guerrero (141) showed that simultaneous treatment of C3H mice with urethan and n-butyl carbamate lead to higher incidence of mammary tumors than when any of the three carbamates was administered separately

Disulfiram has received much attention in recent years as a potential chemo-preventive agent of chemical carcinogenesis. Disulfiram inhibits the carcinogenic action of the following agents of 1,2-dimethylhydrazine and to a lesser extent of azoxymethanol toward the colon of CF_1 mice (227), of bracken fern on the intestines of albino rats (228), of 4-hydroxybutylbutylnitrosamine on the urinary bladder of Wistar rats (229), of 3'-methyl-4-dimethylaminoazobenzene on the liver of Sprague-Dawley rats (230), and of benzo(a)pyrene on the

forestomach of ICR/Ha mice (231). The mechanism of the inhibitory action of Disulfiram has been shown or postulated to be due to inhibition of the metabolic activation of carcinogens (227, 229, 232, 233), inhibition of the binding of carcinogens to macromolecules (231, 233), or to some other as yet unknown mechanism(s). It should be noted, however, that the carcinogenesis-inhibitory activity of Disulfiram is not a general phenomenon. Administration of Disulfiram to Sprague-Dawley rats inhibits the hepatocarcinogenic action of diethylnitrosamine but significantly enhances the induction of esophageal tumors by the agent Similarly, the principal carcinogenicity target organ of dimethylnitrosamine is shifted from the liver to the paranasal sinus by treatment with Disulfiram (169). Moreover, an unusual case of synergism has been reported (234) Feeding Sprague-Dawley rats with Disulfiram and ethylenedibromide resulted in a significant increase in the induction of hemangiosarcoma of the liver, spleen, omentum and kidney, and mammary adenocarcinoma At the doses administered (20 ppm ethylenedibromide in air, 0 05% Disulfiram in diet), neither agent alone was carcinogneic (234).

In addition to Disulfiram, a number of other carbamates and related compounds have been tested for their activity to modify the carcinogenic action of chemical agents. Like Disulfiram, sodium diethyldithiocarbamate and Maneb are effective inhibitors of colon carcinogenesis by 1,2-dimethylhydrazine, whereas Chloropropham is ineffective. The structural requirements for effective inhibition have been discussed by the authors (227, 233). On the other hand, Carbaryl has been found to enhance the induction by benzo(a)pyrene of tumors

in the forestomach of ICR/Ha mice or in the lung of A/J mice The enhancing effect was attributed to ~~the~~ increase of ~~tissue~~ aryl hydrocarbon hydroxylase activity, which is believed to be involved in the metabolic activation of polycyclic aromatic hydrocarbons (250)

The effect of a variety of inducers and inhibitors of microsomal mixed-function oxidase (MFO) on the carcinogenicity of urethan has been tested in an attempt to delineate role of the MFO system in the activation or detoxification of the carcinogen. Phenobarbital, in particular, has been studied by several groups of investigators (223, 235-237) and was found consistently to inhibit urethan-induced lung carcinogenesis in various strains of mice. Other MFO inducers such as chlordane (223), β-naphthoflavone (223), chlordiazepoxide (238), and nikethamide (238) have been found to have the same effect. In contrast, phenothiazine, a MFO inducer under some conditions, was without effect (223). Diethylaminoethyl diphenyl valerate (SKF-525A), a well known inhibitor of MFO, also failed to affect the pulmonary carcinogenic yield of urethan in Swiss (239) as well as A/J mice (223).

Consistent with the finding of the enhancement of urethan carcinogenicity by partial hepatectomy, two chemicals known to stimulate cell proliferation also display similar activity. Witschi et al. (240) showed that butylated hydroxytoluene (BHT), an antioxidant used as food additive, produced proliferation of alveolar cells of the lung of Swiss-Webster and A/J mice Repeated stimulation of cell proliferation by BHT beginning 7 days after urethan treatment significantly enhanced the yield of lung tumors The level of BHT (equivalent to 300 mg/kg

body weight) used was reported to be 100-10,000 times higher than that permitted in the food. The enhancing effect of BHT has been confirmed by Bojan et al. (241) using CFLP mice. However, in contrast to the above study, the BHT effect was significant only if administered 6 or 7 days prior to urethan treatment. The reason for this discrepancy is not known. In addition to BHT, the herbicide, Paraquat (1,1'-dimethyl-4,4'-dipyridylium dichloride), also stimulates the proliferation of lung cells and enhances urethan-induced lung tumorigenesis (241).

A number of general inhibitors of nucleic acid and protein synthesis have been investigated regarding their potential role in modifying the carcinogenicity of urethan. Shimkin et al. (242) found that treatment of A/Jax mice with actinomycin D, puromycin, cytosine arabinoside or 5-fluorouracil has no effect on the incidence of urethan-induced lung tumors. The mean number of tumors per mouse was slightly reduced by actinomycin D, puromycin and cytosine arabinoside and enhanced by 5-fluorouracil, but none of these differences was statistically significant The lack of modifying effect of actinomycin D and puromycin was confirmed by Yamamoto et al (223) who, in addition, showed that cycloheximide, an inhibitor of cytoplasmic protein synthesis, was ineffective. In contrast, chloramphenicol, an inhibitor of mitochondrial protein synthesis, was reported to inhibit the induction of lung tumors in BALB/c and strain A mice by urethan (243) It is not known if the two effects are related. It was suggested (243) that the protective action of chloramphenicol against urethan could be mediated via its influence on enzyme systems or on binding of the carcinogen to cellular macromolecules.

Caffeine has recently gained wide recognition as an inhibitor of error--prone post-replication DNA repair synthesis. Nomura (60, 244) reported unpublished data that caffeine given after urethan treatment suppresses the induction of lung tumors in ICR-JCL mice. The suppressing effect was attributed to the inhibition of post-replication DNA repair, thus resulting in increased cell death. Presumably, some of these cells could have produced a tumor if they had survived. The suppressing effect of caffeine has been confirmed by Theiss and Shimkin (245) using strain A mice However, the most pronounced effect was observed when caffeine was given in two doses 3 hr. before and 3 hr. after urethan treatment. The spontaneous incidence of lung tumors in this strain was also suppressed by caffeine The authors (245) suggested that the anticarcinogenic effect of caffeine against urethan was likely to be due to a general suppression of lung DNA synthesis (rather than post-replication DNA repair). Alternatively, caffeine could exert protective effect by binding to urethan, thus decreasing the likelikhood of binding to lung DNA.

Colchicine, a well known mitotic inhibitor, when given one week or one day before urethan treatment has no effect on the induction of lung tumors in Swiss and strains A and C mice (187) In a more recent study colchicine was administered to ICR mice 24, 9 or 5 hr prior to urethan initiation and TPA promotion Berenblum and Armuth (246) . Significantly higher total incidences of skin tumors and a greater number of malignant tumors were found only in the group receiving colchicine 9 hr before urethan, corresponding to the peak of metaphase arrest It was postulated (246) that the most sensitive

period of tumor initiation occurred during the M phase of the cell cycle (see Suppletory Note) and that colchicine treatment caused the accumulation of cells at metaphase, thus enhancing the incidence of initiation and the eventual development of skin tumors. It should be noted that the exact phase of cell cycle when initiation takes place is a question of great controversy Conflicting results have been presented by various investigators, other phases such as G_2, S, and G_1-S boundary have all been proposed as the most sensitive period.

In addition to the above compounds, the effect of butylated hydroxyanisole (BHA), an antioxidant food additive, on the carcinogenicity of urethan has been tested in A/Hej mice (247). Unlike BHT, BHA was found to reduce the lung tumor yield, the average number of tumors per mouse was reduced from 12.3 to 2.7 after BHA treatment (247). The mechanism of action has not been explored. Sulfanilamide is another compound that has been shown to exert an inhibitory effect on urethan-induced lung tumors Both the incidences and multiplicity of lung tumors in Swiss mice were substantially reduced by the agent (235). When tested under similar conditions, p-aminobenzoic acid was without effect (235).

Viral Interaction. Viral infection may play an important role in the alteration of host susceptibility to chemical carcinogens The viral effect is apparently variable and may be specific for the type of virus involved. Imagawa et al. (248) reported in a 1957 abstract that mice exposed to urethan i.p and influenza A (PRS strain) virus intranasally developed more lung tumors than

control animals receiving either agent alone. Casazza et al. (249) were, however, unable to detect any synergistic action between influenza A_2 virus and urethan Similarly, Stomskaya (250) showed that the incidence of lung tumors in mice that received a combined treatment of myeloid chloroleukemia virus and urethan was not significantly different from the group receiving urethan only. Germ-free BALB/c mice were reported to develop fewer urethan-induced lung tumors than did conventional BALB/c mice (251). The most striking difference between the two sub-colonies of mice was the frequent viral infection (particularly pneumonia virus of mouse and Sendai virus) of the conventional mice and the lack of such infection in the germ-free mice There is no evidence, however, to directly link the viral infection to the greater susceptibility to urethan. A suppression of urethan-induced lung carcinogenesis was observed when strain A/He mice were treated with the Maloney strain of murine sarcoma virus (252). The suppressive effect of the virus could be diminished by increasing the urethan dose The investigators (252) attributed the viral effect to its immunostimulatory activity. A somewhat more complex pattern of interaction between reovirus type 3 and urethan has been demonstrated by Theiss et al. (253) using strain A/St mice When mice were subjected to a single exposure of an aerosol containing the virus either 6 days before, on the same day as, or 14 days after urethan treatment, suppression of lung carcinogenesis of the order of 30-60% was observed. However, when mice were repeatedly exposed to the virus, a slight enhancement of urethan carcinogenicity was observed The authors (253) hypothesized that a single viral exposure was

immunostimulatory through a "rebound" effect, whereas continuous exposure led to immunosuppression, thus inhibiting and enhancing the carcinogenicity of urethan, respectively.

Radiation and Other Physical Factors. The interaction of X-radiation and urethan is quite complex. Depending on the dosage and the target organ concerned, X-radiation may exert either an inhibitory, an enhancing, or no effect on the carcinogenicity of urethan. In general, when given at high doses which inhibit cell division in the lung, X-radiation usually inhibits the development of urethan-induced lung carcinogenesis in mice (see rev ref. 254) For adult (C57L X A)F_1 mice, the inhibitory doses required range from 500-900r (255) The inhibitory effect was abolished if the thorax was shielded during irradiation (256). In contrast to the inhibition to lung carcinogenesis, X-radiation increases the leukemogenic action of urethan in mice, the enhancing effect is particularly evident at low X-ray doses. Kawamoto et al. (191) observed substantial increase in the incidence of leukemia after exposing mice to urethan and to low doses (11 x 40 r or 4x90 r) of X-ray. Berenblum and Trainin (257) demonstrated that the enhancing effect of combined treatment with X-ray and urethan could be observed only if urethan was given after irradiation. On the other hand, Foley and Cole (256), observed a synergistic action whether urethan was given before or after X-ray treatment. Vesselinovitch et al. (258) treated infant mice with low doses of urethan and observed that such treatment enhanced the leukemogenic action of X-radiation given on the 42nd day of life More recently, Myers (127) studied the interaction of X-ray (5 x 165r) and

urethan (5 x 0.9 g/kg) in 3 strains (Sprague-Dawley, Long-Evans, and Collip) of rats At the dosages used, the overall effect of the combined treatment in the induction of mammary, skin, lymphatic and various types of tumors was less than the sum of their separate effects. Clearly additive effect was found only in the induction of lymphatic neoplasms in Collip rats.

Related to X-radiation, inhalation of plutonium dioxide ($^{239}PuO_2$, an α-emitter) 2 weeks before or after urethan treatment to A2G mice reduced the yield of lung tumors (259) The inhibitory effect was attributed to the cell damaged caused by α-irradiation.

Atmospheric pressure and oxygen concentrations may also affect the carcinogenicity of urethan. Mori-Chavez (260, 261) compared the incidences of urethan-induced lung tumors in mice kept at high altitudes and at sea level. High incidences were associated with mice kept at high altitudes, he concluded that lower atmospheric pressure enhanced the susceptibility to lung carcinogenesis. This observation could not be confirmed by Ellis et al. (262) who simulated high altitudes by keeping mice in decompression chambers The treatment reduced rather than enhanced the susceptibility of strain A and A/Grb mice to the carcinogenic action of urethan. The C57Bl mice were unaffected DiPaolo (178) investigated the effect of oxygen concentration on the transplacental carcinogenicity of urethan in strain A mice. Offspring from mothers kept at hyperoxic (100% oxygen) and hypoxid (10% oxygen) conditions developed more lung tumors than those from mothers kept in room air.

5.2 1 6.4 Metabolism and Mechanism of Action.

Urethan Despite decades of research and the relatively simple chemical structure, the metabolic activation and the mechanism of carcinogenic action

of urethan remain obscure. It is generally accepted that cellular metabolism plays a dual role in determining the biological action of urethan, by both catabolizing or activating the compound. The literature on the catabolism of urethan has been thoroughly reviewed by Mirvish (21) in 1968, no major findings have been reported since then. Essentially, hydrolysis seems to be the major route of urethan catabolism with the release of ethanol, carbon dioxide and ammonia (289)

$$NH_2COOC_2H_5 \xrightarrow{\text{esterases}} C_2H_5OH + CO_2 + NH_3$$

Hepatic microsomal esterases appear to be the principal enzyme system involved (21, 290). The rate of catabolism apparently determines the length of time urethan remains in the body which may be a critical factor in determining its carcinogenic potential. The rate of catabolism of urethan in newborn SWR mice, for example, was shown (194) to be 1/10 of that of adults The rate increased slowly for the first 10 days of postnatal life and then sharply between the 15th and 20th day. As it has been discussed in Sections 6.2.1 6 3.-2 and 6.2.1.6.3.9, newborn and infant animals are much more susceptible to the carcinogenic action of urethan than the adults; the lower catabolic rate is most likely a major factor The rate of urethan elimination (which includes catabolism and urinary excretion) from the blood of adult Swiss mice (291, 292), rats (293), rabbits (294) and humans (295) was estimated to be around 60, 50, 25 and 4 mg/ml blood/hr, respectively These data could suggest that urethan might have a longer retention time and possibly greater carcinogenic action in

humans, however, this projection must at best be considered a matter of conjecture in the absence of supportive inter-species correlation studies.

There is general consensus that the carcinogenic action of urethan is mediated *via* an active metabolite (rev. 296). The identity of the active metabolite ("ultimate carcinogen") has, however, remained elusive. Research efforts in the mid-1960's (rev. 21) were mainly directed at investigating the role of N-hydroxyurethan in the metabolic activation of urethan. N-Hydroxyurethan has a carcinogenic potency comparable to that of urethan (*see* Table CXVIII), is chemically more reactive than urethan (21) and has been identified as a minor *in vivo* metabolite of urethan (297, 298) In analogy to the well established role of N-hydroxylation in the activation of carcinogenic aromatic amines (*see* Volume IIB), N-hydroxylation was also suggested to be the initial step in the metabolic activation of urethan (17, 288, 298). This attractive hypothesis was, however, not supported by the investigations of Mirvish (299,300) which revealed that the reverse was probably true, *i.e.*, N-hydroxyurethan more likely acquires carcinogenic properties *via* conversion to urethan. N-Hydroxyurethan is readily reduced to urethan by an enzyme system which is inhibited by SKF-525A, a well known inhibitor of the microsomal mixed-function oxidase (MFO) system In newborn mice, N-hydroxyurethan is also readily converted to urethan, whereas the reverse conversion was not detected (284). Furthermore, Kaye and Trainin (239) showed that the carcinogenic action of N-hydroxyurethan (but not urethan) could be inhibited by treatment of mice with SKF-525A, supporting the conclusion of Mirvish (299, 300) that N-hydroxyurethan must first be converted to urethan to exert carcinogenic action.

The search for the actual active metabolite of urethan was not pursued until recently. Intrigued by reports of potent carcinogenicity of vinyl chloride (see Section 5 2.2.1) and demonstration of incorporation of the radiolabel from labeled ethyl group (but not from ^{14}C-carbonyl or ^{18}O-ethoxy) of urethan into cellular macromolecules (140, 301), Dahl et al. (107) suggested that vinyl carbamate might be a proximate carcinogen of urethan. They proposed (Fig. 30) that vinyl carbamate could be metabolically formed by dehydrogenation The proximate carcinogen thus formed may undergo epoxidation to form a reactive electrophilic ultimate carcinogen This hypothesis is supported by the finding that vinyl carbamate is more carcinogenic and mutagenic than urethan. Vinyl carbamate has been shown to be 10-50 times more active in the initiation of skin tumors and in the induction of lung tumors in mice. The compound is mutagenic in Salmonella test in the presence of activation system, whereas urethan is not (see Section 5.2.1.6.2.2). The activation of vinyl carbamate can be inhibited by typical MFO inhibitors such as DPEA or SKF-525A, although it is not inducible by phenobarbital or 3-methylcholanthrene pretreatment The hypothesis is, however, not supported by the failure of various attempts to detect vinyl carbamate as an in vivo metabolite of urethan. It is possible that vinyl carbamate may be formed at an in vivo enzymatic site and immediately further metabolized. Alternatively, vinyl carbamate may not be a metabolite of urethan, instead, both compounds may be metabolized by a common pathway to an unknown ultimate carcinogenic form ("X") that binds covalently to macromolecules

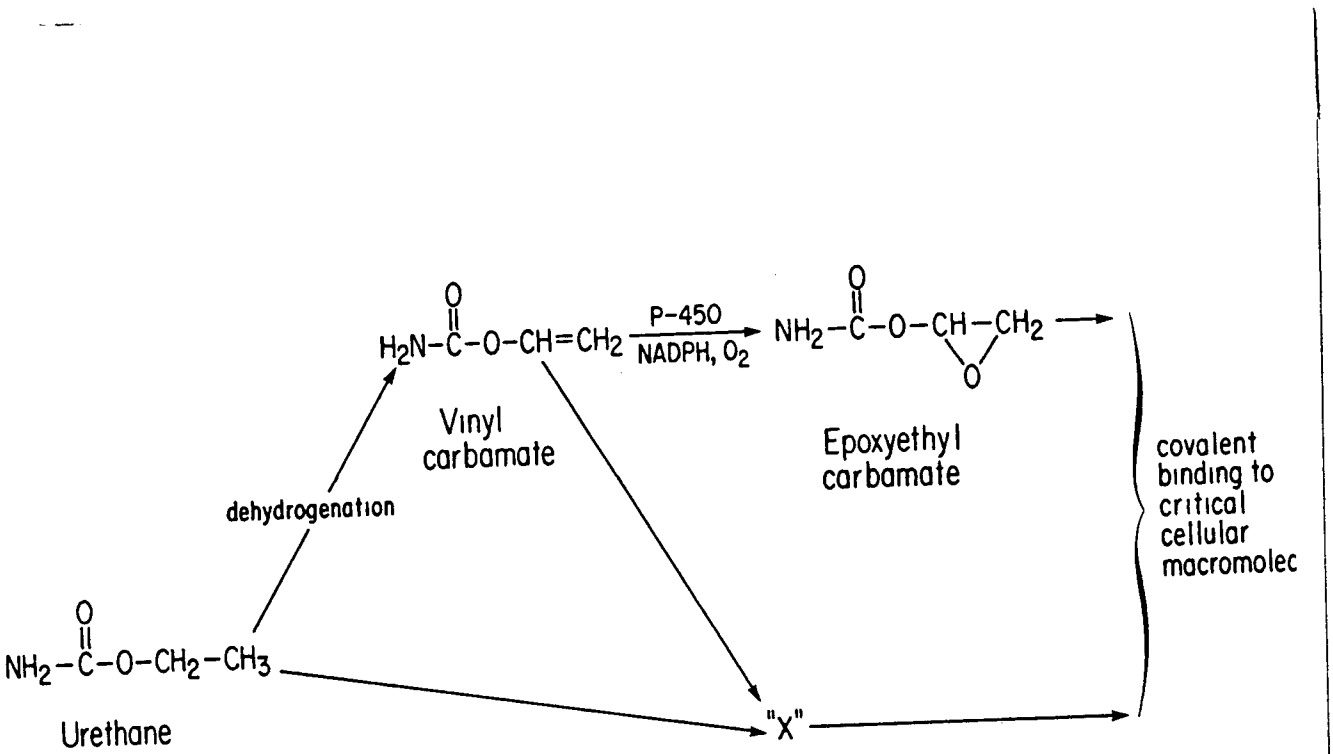

P-450
NADPH, O2
Vinyl
carbamate
Epoxyethyl
carbamate
dehydrogenation
covalent
binding to
critical
cellular
macromolec
"X"
Urethane

Figure 30

LEGEND TO FIGURE 30

Fig. 30. Proposed possible mechanism of metabolic activation of urethan. [Adapted from G A. Dahl, J.A. Miller and E.C. Miller, Cancer Res. 38, 3793 (1978).]

It is now generally believed that the great majority of chemical carcinogens (or their active metabolites) initiate carcinogenesis by binding covalently to critical cellular macromolecules. The in vivo covalent binding of urethan metabolite(s) to macromolecules in target tissues has been demonstrated by various investigators (107, 140, 200, 273, 301-310). Studies by Lawson and Pound demonstrated that covalent binding of urethan metabolite to liver DNA occurres to a greater extent than that to RNA or cell protein (305) Partial hepatectomy, which is known to enhance the susceptibility of liver to the carcinogenic action of urethan, increases the level of binding to liver DNA, without significantly affecting the binding to lung or epidermal DNA (200, 306, 307). The greater susceptibility of male mice to urethan-induced liver carcinogenesis is reflected by the higher level of binding, no such sex difference is observed in the binding to lung or epidermal DNA (200). Comparison of the binding of urethan and its homologs or N-alkyl derivatives (which are either weaker carcinogens or inactive) to liver epidermal DNA indicate that urethan is the most active compound and urethan binding persists significantly longer than other carbamates (273) The persistence of urethan binding to epidermal DNA is even further prolonged by croton oil treatment, which is known to promote skin carcinogenesis by urethan (273)

The possible role of RNA binding in the initiation of urethan carcinogenesis has been investigated by Williams and associates (296, 302, 310). Administration of radioactively labeled urethan to mice brought about substantial incorporation of the radioactivity to liver RNA Most of the radioactivity was present

in a single compound, ethyl cytosine-5-carboxylate. No such preferential incorporation occurred in control animals receiving radioactively labeled ethanol or sodium bicarbonate (302). The extent of this preferential incorporation parallels the activity of RNA synthesis (310). Based on some suggestive evidence that the carcinogenic activity is greatest if administered during the peak of RNA synthesis, Williams and coworkers (296, 310) hypothesized that the presence of ethyl cytosine-5-carboxylate in RNA could play a role in urethan carcinogenesis. This hypothesis was, however, considerably weakened by the demonstration (310) that *in vivo* labeling of RNA also occurred with noncarcinogenic methyl carbamate Furthermore, it is not known whether the formation of ethyl cytosine-5-carboxylate occurred as a result of direct binding of the urethan metabolite to RNA or indirect incorporation of pre-formed nucleoside analog into RNA.

As might be expected from the inadequate knowledge of the ultimate-carcinogenic form of urethan, the nature of urethan binding to macromolecules has not been clearly elucidated The isolation of labeled ethyl cytosine-5-carboxylate from liver RNA of mice treated with urethan, labeled with ^{14}C at the carboxy (or carbonyl) position, indicated that the entire ethoxycarbonyl residue may be bound to RNA (302), however, it could not be ascertained whether the formation of the adduct occurs as a result of direct binding of urethan metabolite to RNA (310). The presence of some unidentified radioactively labeled components in DNA and RNA of rats administered of [1-^{14}C]-urethan or [2-^{3}H]-urethan suggested that labeling of the ethyl residue alone was sufficient to demonstrate

the binding (303). Lawson and Pound (301) compared the binding of NH_2-CO--O-$^{14}CH_2$-CH_3 and NH_2-^{14}CO-O-CH_2-CH_3 to mouse liver DNA and concluded that the carbonyl (or carboxyl) carbon was not involved in the binding. The conclusive evidence that only the ethyl residue is involved, was furnished in the study using NH_2-CO-^{18}O-CH_2-CH_3. No ^{18}O enrichment was detected in the oxygen of liver DNA, indicating that only the ethyl residue, not the ethoxy residue, was involved in the binding (140). Chromatographic analysis of acid hydrolysates of liver DNA from mice treated with [1-^{14}C]urethan provided no evidence of alkylation of purine or pyrimidine bases. Various hydrolytic and enzymatic treatments revealed that ^{14}C was bound in an alkyl group as an ester to a phosphate group in the DNA chain Enzymatic release of the alkyl group yielded a volatile compound identified as ethanol (140). Thus, it appears that only the ethyl residue is involved in the binding and the site of binding is mainly at the phosphate backbone. A different conclusion, however, has been recently reached by Dahl et al (107). These investigators compared the level of binding of ^3H and ^{14}C to hepatic DNA, rRNA and protein of mice given [1-^{14}C]urethan and[1,2-^{3}H]urethan and found that the $^3H/^{14}C$ ratio of the adduct was substantially lower than that of the urethan administered. They postulated that the ethyl residue must be dehydrogenated in vivo to vinyl carbamate (thus losing its tritium label) before binding to macromolecules possibly in the form of an epoxide.

In addition to nucleic acid binding, modification of cell proteins by urethan has also been reported A recent study by Gronow and Lewis (311) indicated

that a protein fraction of non-histone protein of mouse liver may be modified (as measured by isoelectric focusing electrophoresis) after a single carcinogenic dose of urethan to suckling mice. This finding may be of potential significance in urethan carcinogenesis in view of the increasing evidence that non-histone proteins play a crucial role in the control of gene transcription.

Diaryl Acetylenic Carbamates. A number of diaryl acetylenic carbamates have been shown to be potent carcinogens (see Section 5.2.1 6.3.3). Based on structural considerations, these compounds could conceivably alkylate tissue nucleophiles directly by substitution reactions involving loss of carbamate anion Such an electrophilic activity was indeed demonstrated by Sharpe et al. (25) using methionine or guanosine as nucleophiles Incubation of several acetylenic carbamates with ^{3}H-labeled methionine or ^{14}C-labeled guanosine yielded reaction products. 1,1-Diphenyl-2-butynyl N-cyclohexylcarbamate and 1-phenyl-1-(3,4-xylyl)-2-propynyl N-cyclohexyl carbamate were the most reactive compounds in this assay. Mass spectral analysis of a major product between 1,1-diphenyl-2-butynyl N-cyclohexyl carbamate and methionine was consistent with the formation of 1-methylmercapto-1,1-diphenyl-2-butyne. The direct-acting alkylating activity of these compounds is consistent with their direct-acting mutagenic activity (see Section 5.2.1.6 2.2) Tumor induction may, however, occur at site(s) distant from the site of administration.

Carbamate Pesticides The metabolism of carbamate pesticides has been a subject of extensive research in the last decade, a number of excellent reviews on this topic have been published in recent years (3, 312-316) Essentially, oxidation, conjugation and hydrolysis are the three principal types of

metabolic reactions that carbamate pesticides may undergo in living organisms. Oxidation, generally involving microsomal mixed-function oxidase (MFO), is the most important route. Depending on the functional groups present in the molecule, a variety of reactions, including aliphatic hydroxylation, aromatic hydroxylation, sulfoxidation, and N-, O- and possibly S-dealkylation (as depicted in Fig. 31) may occur. Fig. 31 Carbamate pesticides and metabolites containing such functional groups as epoxide, hydroxyl, amino, carboxyl or sulfhydryl may be readily conjugated, predominantly with the formation of sulfates, glucuronides and mercapturic acids (the latter derived from glutathione conjugates). Carbamate pesticides may also be metabolized by esterase-catalyzed hydrolysis; however, depending on the compound and animal species involved, the hydrolytic route may be only of minor importance. With some exceptions, most of the above-mentioned metabolic pathways are detoxifying in nature, in the following paragraphs, only the reactions that are of potential significance in the generation of mutagenic or carcinogenic intermediates are discussed.

Carbaryl is the most extensively studied carbamate insecticide. The metabolism of this compound has been reviewed (3, 312-314), the proposed metabolic pathways are shown in Fig. 32. Fig. 32 Although not actually identified, the formation of 3,4- or 5,6-epoxides as intermediates in the metabolism of carbaryl is implicit from the known end products. These epoxides are highly reactive electrophiles and can conceivably bind covalently to nucleophilic sites in cell macromolecules. The in vitro covalent binding of carbaryl was indeed demonstrated by Oonithan and Casida (317). Incubation of rat liver microsomes with

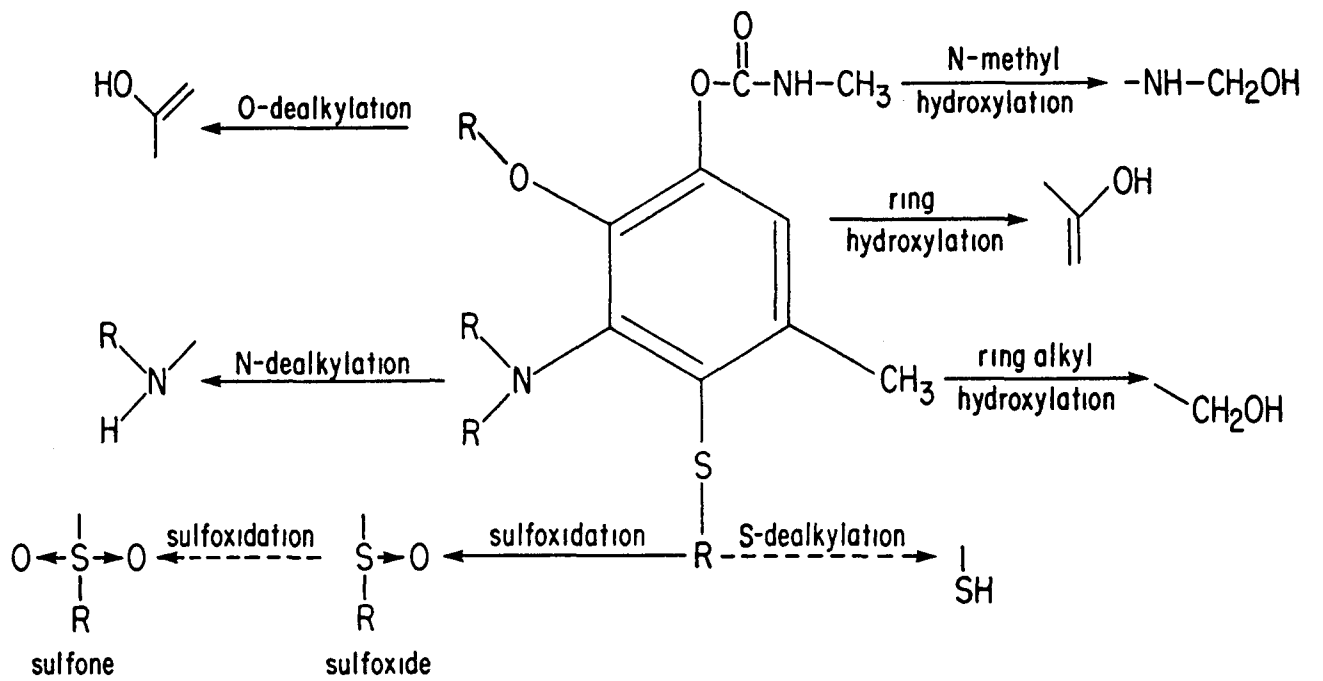

HO
O-dealkylation
O
O-C-NH-CH3
N-methyl
hydroxylation
-NH-CH2OH
R
O
ring
hydroxylation
OH
R
N
H
N-dealkylation
R
N
R
CH3
ring alkyl
hydroxylation
CH2OH
S
O
S
O
sulfoxidation
O
S
O
sulfoxidation
R
S-dealkylation
SH
R
R
sulfone
sulfoxide

Figure 31

LEGEND TO FIGURE 31

Fig. 31 Possible sites of oxidation on a hypothetical N-methyl-aromatic carbamate pesticide. [Modified from R.J. Kuhr and H.W. Dorough, "Carbamate Insecticides Chemistry, Biochemistry and Toxicology", CRC Press, Cleveland, Ohio, 1976.]

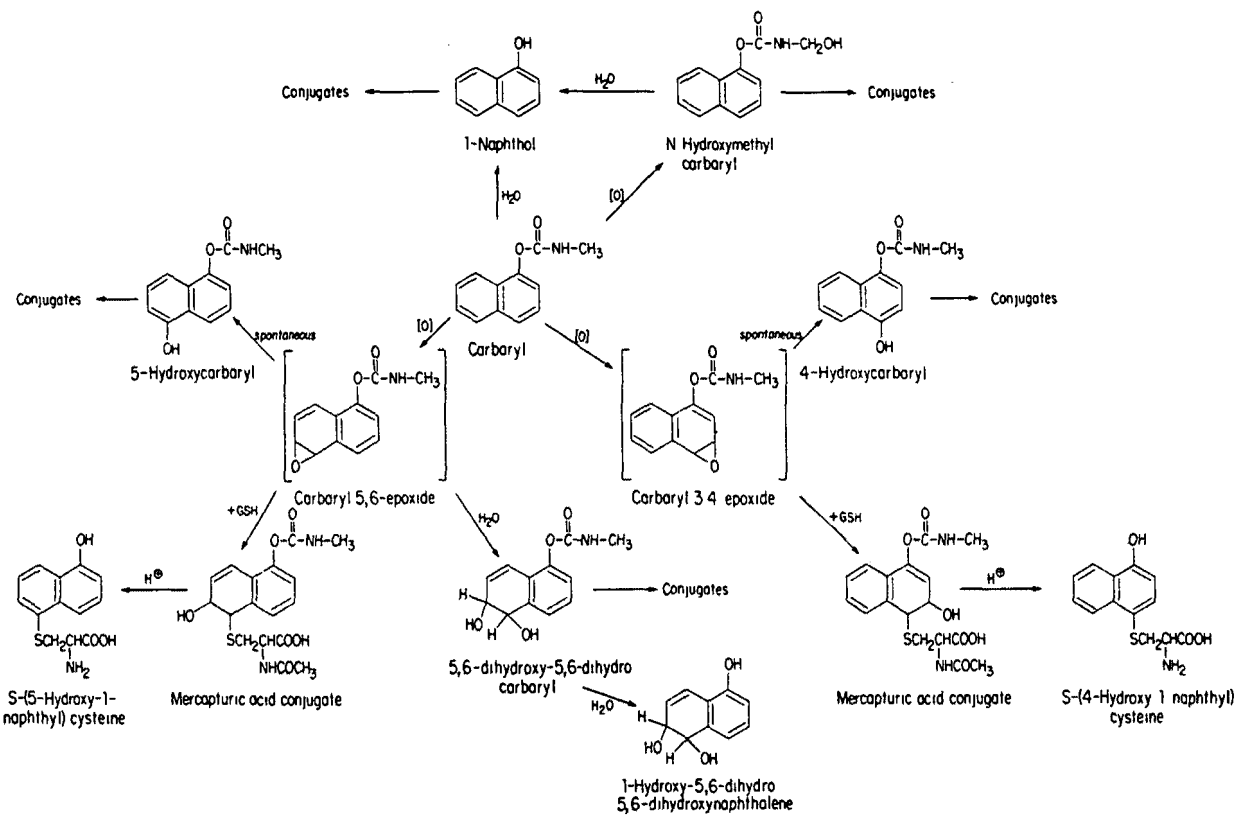

Conjugates
1-Naphthol
H_2O
N Hydroxymethyl carbaryl
Conjugates
H_2O
[O]
Conjugates
5-Hydroxycarbaryl
spontaneous
[O]
Carbaryl
[O]
spontaneous
4-Hydroxycarbaryl
Conjugates
Carbaryl 5,6-epoxide
Carbaryl 3 4 epoxide
+GSH
H_2O
+GSH
$H^{\oplus}$
$H^{\oplus}$
Conjugates
S-(5-Hydroxy-1-naphthyl) cysteine
Mercapturic acid conjugate
5,6-dihydroxy-5,6-dihydro carbaryl
Mercapturic acid conjugate
S-(4-Hydroxy 1 naphthyl) cysteine
H_2O
1-Hydroxy-5,6-dihydro 5,6-dihydroxynaphthalene

Figure 32

LEGEND TO FIGURE 32

Fig. 32. Proposed metabolic pathways of Carbaryl.

naphthyl-^{14}C carbaryl in the presence of NADPH led to significant incorporation of radioactivity into microsomal proteins This finding has recently been confirmed by Miller et al. (318) Carbaryl with ^{14}C-labeled N-methyl group did not bind to microsomes indicating that only the aromatic ring was involved in the binding. The binding reaction is catalyzed by microsomal MFO (indicated by the dependence on NAPDH and oxygen; inhibition by nitrogen, carbon monoxide, SKF-525A, and stimulation by phenobarbital or 3-methylcholanthrene pretreatment) and is inhibited by glutathione or cystein The biological significance of this finding remains to be elucidated.

Zectran (Mexacarbate) is one of the very few carbamate pesticides for which there is some (although not convincing) evidence of potential carcinogenicity (see Section 5.2.1.6.3.6). Very little information is available on the metabolism of Zectran in mammals (3, 313, 315), the principal metabolites are shown in Fig 33. Zectran (i) is readily metabolized, predominantly by hydrolysis, when administered orally to mice (319). Almost 70% of ^{14}C from ^{14}C-carbonyl-labeled Zectran is expired as $^{14}CO_2$ within 6 hours (319) The nature of the radioactively labeled metabolites in the urine of dogs given ring-3-^{14}C-methyl-labeled Zectran was studied (320) The major metabolites were 4-dimethylamino-3, 5-dimethylphenol (ii) and its conjugates, small amounts of conjugated 2, 6-dimethylhydroquinone (iii) were also detected. As many as 9 metabolites were detected in in vitro metabolic studies using rats or human liver microsomes (317, 321-324) The two major metabolites are 4-dimethylamino-3, 5-dimethylphenyl N-hydroxymethylcarbamate (iv) and 4-methylamino-3, 5-dimethylphenyl N-methylcarbamate (v), the minor metabolites include

← Fig. 33.

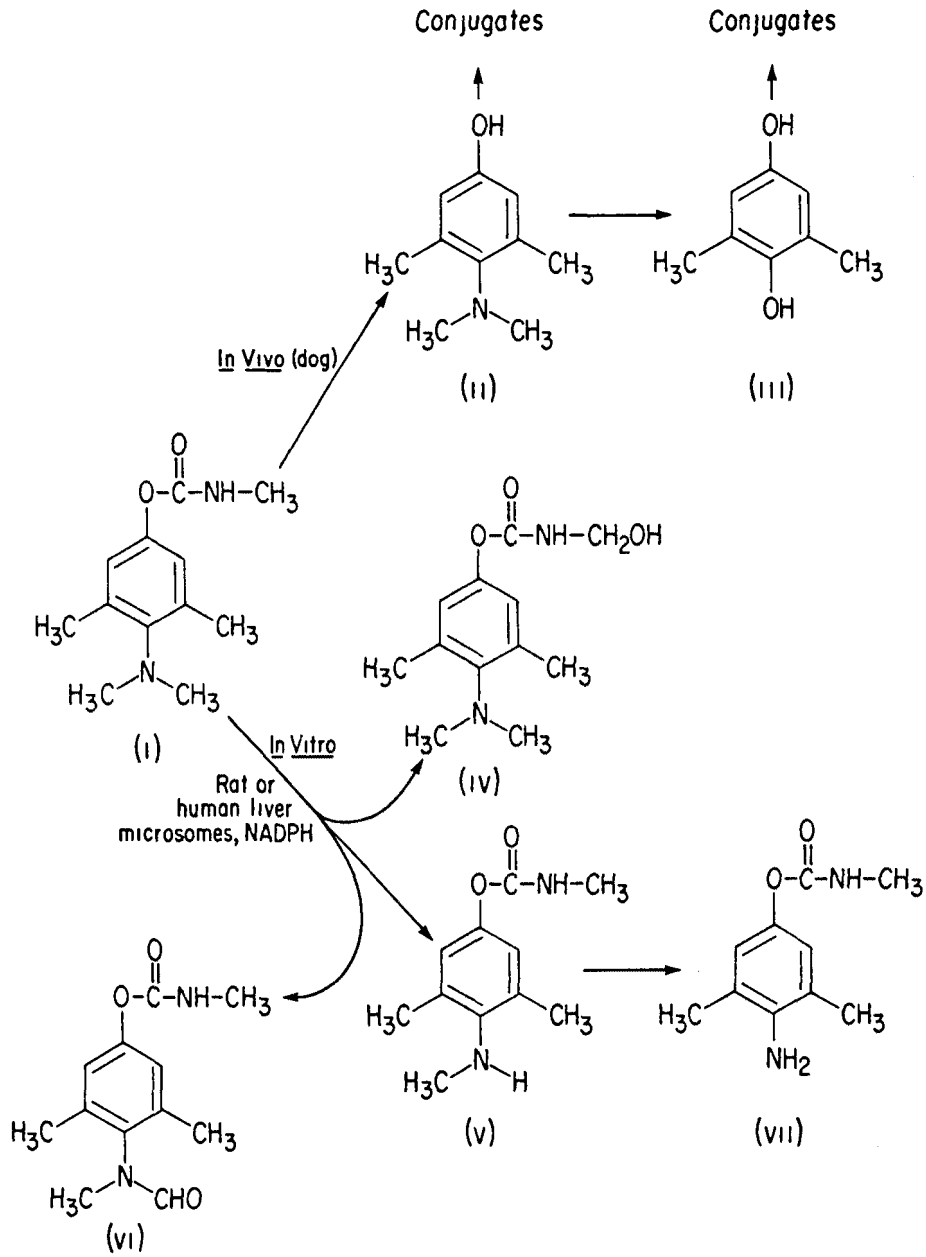
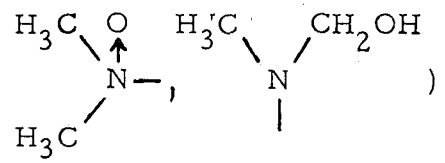

Conjugates
Conjugates
OH
OH
In Vivo (dog)
(II)
(III)
(I)
(IV)
In Vitro
Rat or
human liver
microsomes, NADPH
(V)
(VII)
(VI)
CHO
NH2

Figure 33

LEGEND TO FIGURE 33

Fig 33 Some *in vivo* and *in vitro* metabolites of Zectran. The designations used are *i* = Zectran, *ii* = 4-dimethylamino-3,5-dimethylphenol, *iii* = 2,6-dimethylhydroquinone, *iv* = 4-dimethylamino-3,5-dimethylphenyl N-hydroxymethylcarbamate, *v* = 4-methylamino-3,5-dimethylphenyl N-methyl carbamate, *vi* = 4-methylformamido-3,5-dimethylphenyl N-methylcarbamate, *vii* = 4-amino-3,5-dimethylphenyl N-methylcarbamate.

4-methylformamido-3,5-dimethylphenyl N-methylcarbamate (vi) and 4-amino-3,5-dimethylphenyl N-methylcarbamate (vii). The possible mutagenicity or carcinogenicity, if any, of the above metabolites has not been explored It is possible that unstable reactive intermediates (e g , ) may be formed in the process of N-demethylation of the 4-dimethylamino group Such intermediates were thought to be the reactive intermediates of carcinogenic compounds such as hexamethylphosphoramide (see Section 5 2 1 4 1) 4-Methylformamido-3,5-dimethylphenyl N-methylcarbamate may also be a potentially active metabolite (it is structurally similar to the carcinogen N-methyl-N--formyl-hydrazine) (Section 5.2 1.3 3 2 5)

S-Chloroallyl Thiocarbamates Three S-chloroallyl thiocarbamates (Diallate, Sulfallate, and Triallate) have been shown to be mutagenic (Section 5 2 1 6 2 2) Of these, Diallate and Sulfallate are carcinogenic in rodents, whereas the carcinogenicity of Triallate does not appear to have been studied Mutagenicity studies indicate that metabolic activation is required for consistently demonstrable activity The mechanism of metabolic activation of Diallate has been investigated by Schuphan et al (105) Rats given an oral dose of ^{14}C-carbonyl-labeled Diallate expired $^{14}CO_2$ (20% of the dose) and excreted labeled mercapturic acid conjugate (62% of the dose) and other metabolites The fate of the chloroallyl portion of Diallate was examined by using ^{14}C-allyl-labeled Diallate. Dichloroallylsulfonic acid was the major metabolite in both in vitro and in vivo studies, whereas 2- ^{14}C -chloroacrolein appears to

have been detected only in in vitro studies. Based on these results, the authors (105) proposed that Diallate was first sulfoxidized to Diallate sulfoxide (which is highly reactive and cannot be isolated from the metabolites). Most of the sulfoxide thus formed is then detoxified by reacting with glutathione to yield mercapturic acid conjugate and dichloroallylsulfonic acid as the final metabolites, whereas a portion may undergo spontaneous 2, 3-sigmatropic rearrangement followed by 1, 2-elimination to yield diisopropylcarbamoylsulfenyl chloride and 2-chloroacrolein (Fig 34) The latter pathway has been proposed as the activation mechanism of Diallate. Both 2-chloroacrolein and synthetic Diallate sulfoxide have been shown to be mutagenic without activation The cis-isomer of Diallate, which yields more 2-chloroacrolein than its trans-isomer, also displays greater mutagenicity after metabolism (105) It remains to be investigated whether 2-chloroacrolein is carcinogenic The metabolic activation of Triallate and Sulfallate probably follows a similar mechanism as Diallate (105).

← Fig. 34.

Dialkyldithiocarbamates With a few exceptions, dialkyldithiocarbamates have not been shown to be carcinogenic, although there is some evidence that a variety of dimethyldithiocarbamates (but not their ethyl analogs) are mutagenic without metabolic activation It has not been clearly established whether the carcinogenic action of the active dialkyldithiocarbamates is due to the dialkyldithiocarbamate anion or to the metal ion. The metabolic pathways of dialkyldithiocarbamates (Fig 35) appear to be applicable to both dimethyl (325) as well as

← Fig. 35.

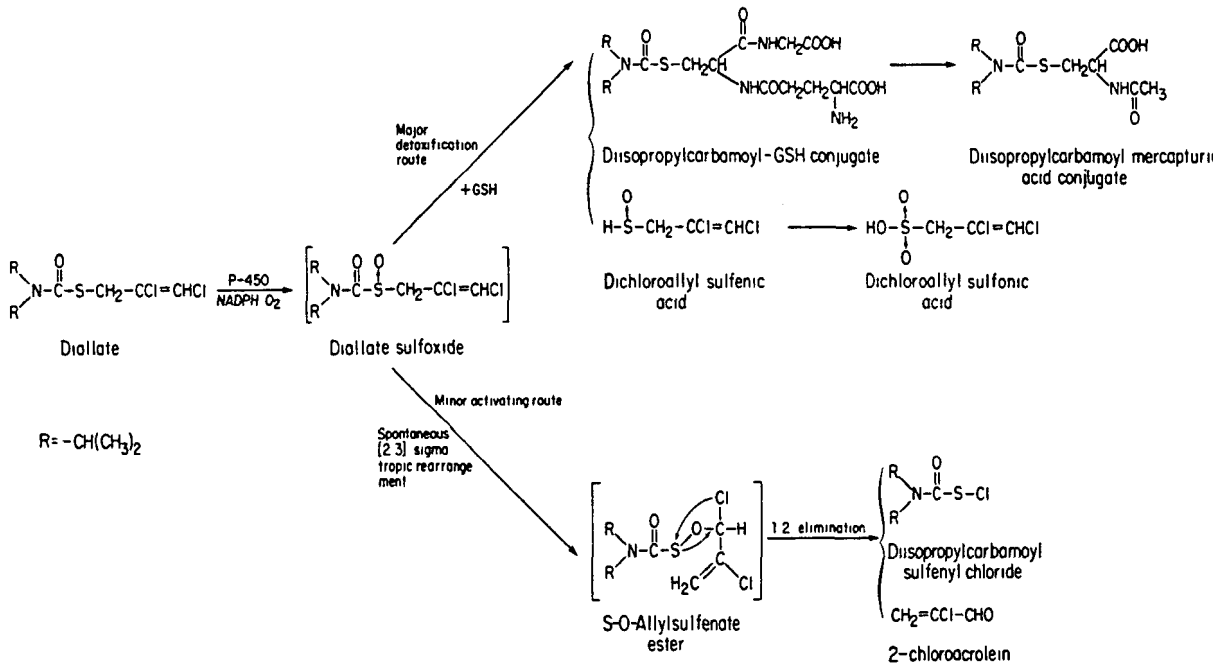

Major detoxification route
+GSH
P-450
NADPH O2
Diallate
Diallate sulfoxide
Diisopropylcarbamoyl-GSH conjugate
Diisopropylcarbamoyl mercapturic acid conjugate
Dichloroallyl sulfenic acid
Dichloroallyl sulfonic acid
Minor activating route
Spontaneous [2 3] sigma tropic rearrangement
R= -CH(CH3)2
1 2 elimination
S-O-Allylsulfenate ester
Diisopropylcarbamoyl sulfenyl chloride
2-chloroacrolein

Figure 34

LEGEND TO FIGURE 34

Fig. 34. Proposed metabolic pathways of Diallate. [Modified from I. Schuphan, J.D. Rosen and J.E. Casida, Science 205, 1013 (1979).]

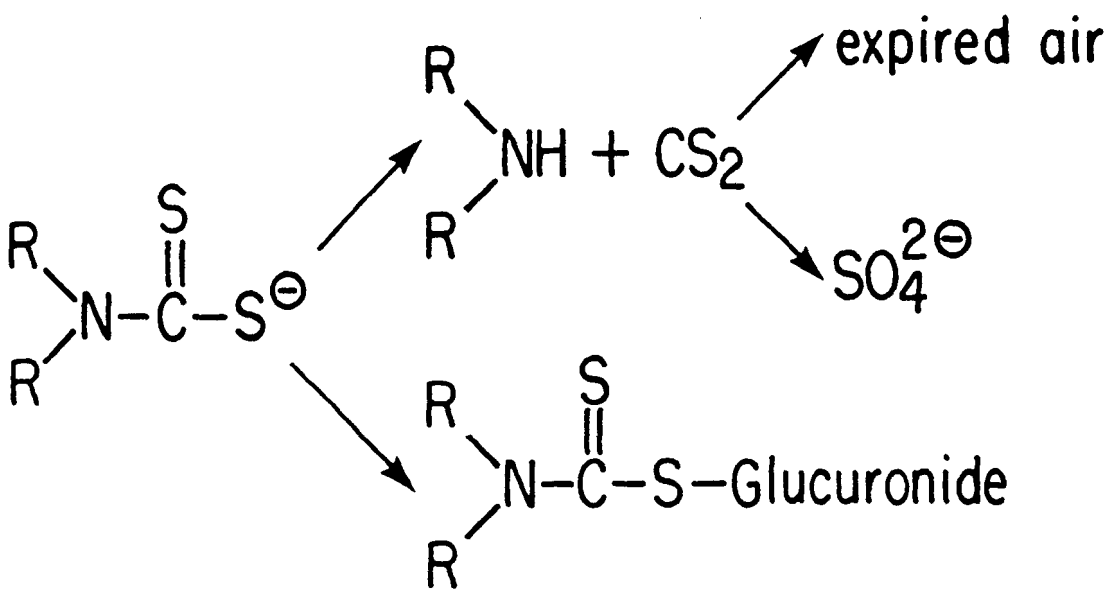


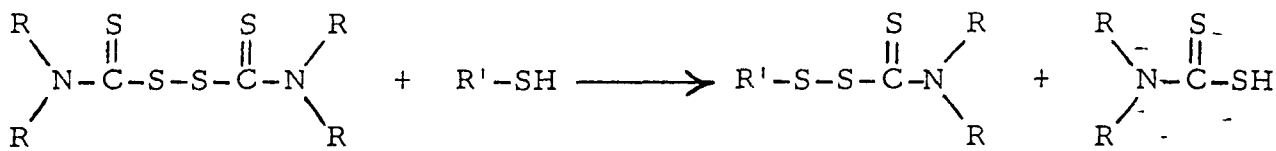
Figure 35

LEGEND TO FIGURE 35

Fig. 35 Metabolic pathways of dialkyldithiocarbamates.

diethyldithiocarbamates (326). None of the metabolites are more reactive than the parent compound Thus, it is possible that the active dialkyldithiocarbamates may exert carcinogenic or mutagenic action by acting directly on target molecules This is supported by the demonstration (45, 46) that dimethyldithiocarbamates are mutagenic as such One possible mechanism of action is the chelation of metals of key enzymes or proteins involved in the control of cell differentiation or gene transcription

Tetraalkylthiuram Disulfides Tetraalkylthiuram disulfides (e g., Thiram, Disulfiram) are metabolized in a manner essentially similar to that of their corresponding dialkyldithiocarbamates (3, 327, 328). The first step involves the conversion of the parent compound, either by free radical process or by nucleophilic substitution reaction on sulfhydryl groups,to form a new mixed sulfide plus a dialkyldithiocarbamate



The dithiocarbamate thus formed is then metabolized, as summarized in Fig 35. Thus, the mutagenic or carcinogenic action of tetraalkylthiuram disulfides may also proceed ~~possibly~~ via direct action on target molecules This is in fact the case for Thiram which induces reversion of base-substitution mutants (TA 100 or 1535) in the Salmonella test without metabolic activation, however, Disulfiram does not appear to be mutagenic. Interestingly the expression of mutagenic activity of Thiram in frame-shift mutagens (TA 98 or 1538) does require metabolic activation (48). It is possible that some active metabolites of

Thiram have yet to be identified. One hypothetical mechanism of action of tetraalkylthiuram disulfide is to act as a cross-linking agent, linking two peptide chains containing free SH groups by disulfide bridge by the substitution reaction depicted above.

Ethylenebisdithiocarbamates. The metabolism of sodium, manganese, and zinc salts of ethylenebisdithiocarbamic acid (Nabam, Maneb, Zineb) has been extensively studied and this topic has been exhaustively reviewed in 1976 (23, 312, 316) A variety of new reports have appeared since then (329-332). ← Fig. 36. The metabolic pathways are depicted in Fig 36 Ethylenebisdithiocarbamate may be metabolized by (a) oxidation to ethylenethiuram disulfide (ETD), possibly mediated in vivo by cytochrome c, (b) degradation of the molecule to release ethylenediamine (EDM) and carbon disulfide, and (c) formation —— after splitting off hydrogen sulfide —— of ethylenebisisocyanato sulfide (EBIS), ethylenebisisothiocyanate (EDIT) and ethylenethiuram monosulfide (ETM). The latter yields ethylenethiourea (ETU) which, in turn, may be oxidized to ethyleneurea (EU). Among these metabolites, ETU has been shown to be carcinogenic and has been considered to be responsible for the carcinogenic action of Maneb and Zineb in some studies, the details of the carcinogenicity of ethylenethiourea will be further discussed in Section 5.2.2.6. It should be noted that, in addition to the metabolites shown in Fig 36, the presence of large amounts of unidentified polar metabolites has been noted (332, 333), the biological activity of these polar metabolites has yet to be elucidated

5 2 1 6 5 Environmental Significance. Urethan has been a compound of considerable environmental concern Possible exposure to this carcinogen

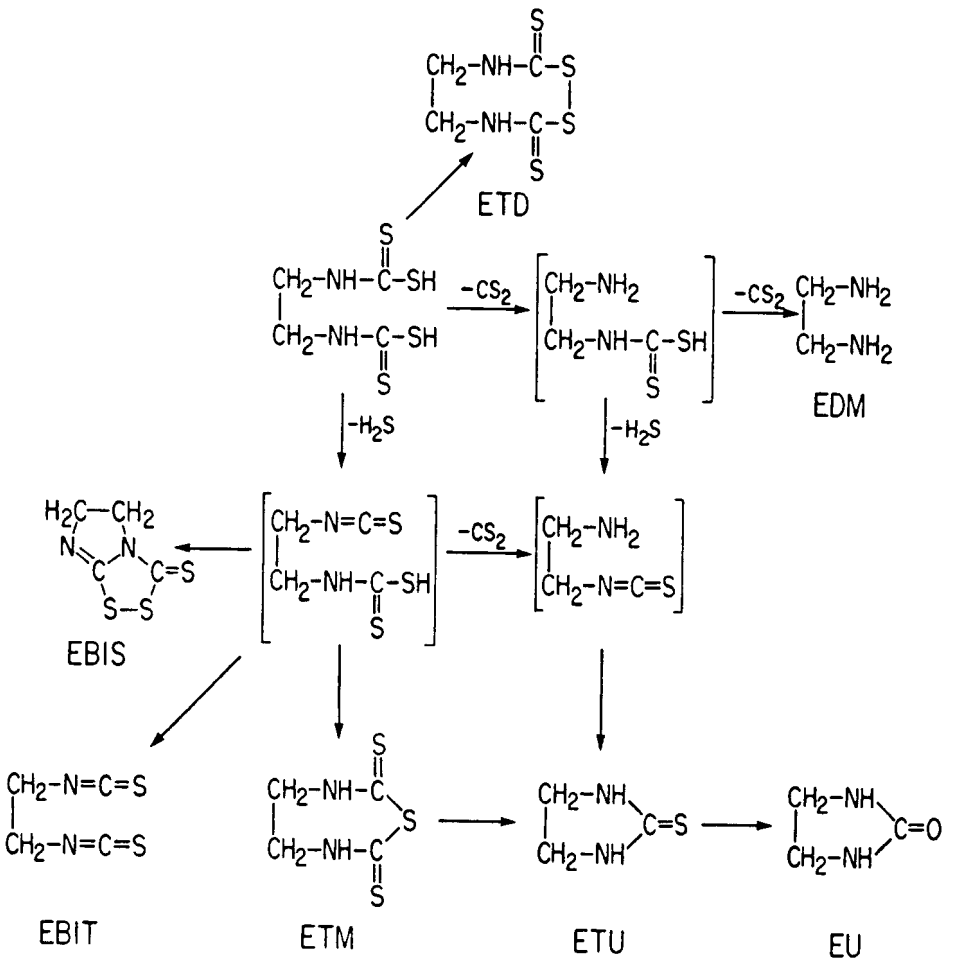


Figure 36

LEGEND TO FIGURE 36

Fig. 36 Proposed metabolic pathways of ethylenebisdithiocarbamates. The chemical names of the metabolites are ETD = ethylenethiuram disulfide, EDM = ethylenediamine; EBIS = ethylenebisisocyanato sulfide, EBIT = ethylenebisisothiocyanate, ETM = ethylenethiuram monosulfide, ETU = ethylenethiourea, EU = ethyleneurea

may occur from pharmaceuticals, industrial chemical intermediates, as well as fermented beverages and foods. Urethan was formerly used in human medicine as an antineoplastic agent (particularly in the treatment of chronic leukemia and multiple myeloma), as a hypnotic or sedative, as a component of a sclerosing solution (together with quinine) for varicose veins, as an antidote to strychnine poisoning, as an adjunct to sulfonamide therapy, and as a topical bactericide (108, 334, 355). There is no evidence that urethan is still being used in the U.S. in human medicine, but its veterinary usage probably still continues. In a 1975 publication of Nomura (336), urethan was reported to be present (as a co-solvent) in four Japanese pharmaceutical products intended for human parenteral use One of these products, "Grelan Injection", when administered to ICR-JCL mice, actually induced lung tumors It is possible that at the present time inventories and stocks of drugs may still carry products containing urethan, as they may have been manufactured before the discontinuation of the use of urethan in human medicine.

Industrially, urethan has not been used in any large amounts in recent years. The annual production in 1977 was estimated to be greater than 1,000 lbs. (337) Urethan has been mainly used as a chemical intermediate in the preparation of amino resins, and as a solubilizer and co-solvent for fumigants, pesticides, and cosmetics The major present use of urethan is believed to be as a chemical intermediate, primarily for reaction with formaldehyde to produce N-hydroxymethyl derivatives useful as cross-linking agents in textile treatment (108, 338)

Urethan has been reported to occur as a result of the reaction of ammonia with diethylpyrocarbonate, an antimicrobial food additive for beverage. By isotope dilution technique, Lofroth and Gejvall (339) estimated that as much as 2 6 mg/l urethan could be produced by the addition of 500 mg/l diethylpyrocarbonate to a white wine at pH 3. 4 and having an estimated ammonia content of 5 mg/l. The urethan yield in beer and orange juice treated with the food additive was of the order of 1. 3 and 0. 17-0 58 mg/l, respectively (339). This has been confirmed by other investigators, but a much lower level (as much as 100 times less) of urethan was found (340-343) In 1972, the U.S. Food and Drug Administration rescinded the permission for the use of diethylpyrocarbonate as an additive in beverages (344). Ough (342) has recently developed a new analytical method for the determination of low levels of urethan in beverages and foods. Most fermented foods and beverages were found to contain urethan ranging from a trace to 6 0 μg/l. A sample of Japanese sake tested contained an exceptionally high level of urethan (154-192 μg/l) No urethan was detected in unfermented food products. The urethan was apparently from natural sources and was postulated to arise as a result of the reaction of ethanol with carbamyl phosphate, the latter could be synthesized from ATP, ammonia and carbon dioxide by yeast. Ough (343) further re-evaluated the extent of urethan formation in commercial wine by the addition of 50-100 mg/l diethylpyrocarbonate and found that the additional amounts of urethan formed were generally less than 1 μg/l. The author called for a re-evaluation of FDA's ~~stand on the~~ ban of the additive

It should be noted that the carcinogenicity of urethan is well established. A dose-response study by Schmähl et al. (345) indicates that a daily intake as low as 100 μg/kg urethan may induce tumors in mice. Furthermore, the demonstration by Goerttler and Lohrke (124) (Section 5.2.1.6 3 8), that low doses of urethan may cross the placental barrier and initiate tumorigenesis in the fetus, as well as the many examples of synergism and enhancement of urethan carcinogenesis by various chemicals (Section 5 2.1 6.3.9) underscore the importance of urethan as a potential environmental carcinogen for humans. ~~present authors' view, the finding of Ough (342, 343) should raise the question of whether one should evaluate the safety of some fermented food products rather than to consider the reinstatement of the use of the controversial food additive. Although seemingly impossible, naturally occurring carcinogens should not be immune from regulation.~~

The environmental significance of other carbamates, thiocarbamates, and substituted urea compounds, has not been critically evaluated. Many of these are used in very large amounts agriculturally and industrially, and could conceivably be a major concern Fortunately, most of these compounds have a relatively low tendency to accumulate in the environment and relatively few have been unequivocally proven to be carcinogenic The major uses and the annual production or consumption data of some of the more important carbamates and related compounds are tabulated in Table CXXX. Essentially, human exposure to these compounds may occur via occupational handling, pharmaceutical use, and ingestion of pesticide residues in food products The

Major Uses and Annual Production or Consumption of Some Important Carbamate, Thiocarbamate and Substituted Urea Compounds

Compound	Major Uses	Annual Production/Consumption[a]
Zectran (Mexacarbate)	Insecticide, molluskicide	$<1,000$ lb (discontinued in 1975)
Propoxur (Baygon)	Insecticide	3×10^5 lb. (1974)
Carbaryl (Sevin)	Insecticide, acaricide, molluskicide	26×10^6 lb. (1974)
Carbofuran (Furadan)	Insecticide, nematocide	12×10^6 lb (1974)
Propham (IPC)	Herbicide	2×10^5 lb (1975)
Chloropropham (CIPC)	Herbicide	$1\ 1 \times 10^6$ lb (1975)
Benomyl (Benlate)	Fungicide	3×10^6 lb. (1975)
Aldicarb (Temik)	Insecticide, miticide, nematocide	1.6×10^6 lb (1974)
Diallate (Avadex)	Pre-emergence herbicide	$1\text{-}5 \times 10^6$ kg (world-wide)
Sodium diethyldithiocarbamate	Chelating agent, rubber vulcanization accelerator, metal poisoning therapy	1×10^6 kg (world-wide)
Ziram	Fungicide, rubber vulcanization accelerator	1.89×10^6 lb (1976)

Ethyl zimate	Rubber vulcanization accelerator, heat stabilizer for polyethylene	0.927×10^6 lb. (1975)
Butyl zimate	Accelerator for latex dispersion and cement, ultraaccelerator for lubricating oil additive	$2\ 92 \times 10^6$ lb. (1976)
Ferbam	Fungicide, rubber vulcanization accelerator	8.0×10^5 lb (1968)
Thiram	Rubber (vulcanization) accelerator, fungicide, animal repellant	5.8×10^6 kg (1975)
Disulfiram	Rubber (vulcanization) accelerator, aversion therapy for chronic alcoholism	$5.1\text{-}5\ 5 \times 10^5$ kg
Maneb	Contact fungicide	5.5×10^6 lb. (1972)
Zineb	Fungicide	1.4×10^6 lb (1968)
Carbromal	Sedative, hypnotic	——
Monuron	Herbicide on non-crop land	$2\ 3\text{-}4.0 \times 10^5$ kg (1973)

[a]Except where specified, the production/consumption data are those of the U.S. Numbers in parentheses indicate the year the data were obtained Source of information SRI, "A Study of Industrial Data on Candidate Chemicals for Testing", EPA 560/5-77-006, Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, 1977, IARC Monog No 12, International Agency for Research on Cancer, Lyon, France, 1976, and information dossiers prepared for the U.S. Environmental Protection Agency, Washington, DC.

threshold limit values (TLVs) for occupational exposure adopted by the American Conference of Governmental and Industrial Hygienists for a number of carbamates and related compounds are as follows Propoxur, 0.5 mg/m^3, Carbaryl, 5 mg/m^3, Carbofuran, 0 1 mg/m^3, Benomyl, 10 mg/m^3 (proposed), Thiram, 5 mg/m^3, Disulfiram, 2 mg/m^3 (157) As might be expected from the heavy use of carbamates and related compounds as pesticides in crop protection, the presence of residual amounts of these compounds in harvested foods appears to be inevitable although there is a paucity of quantitative data Many recent articles have discussed the problems of measurement, degradation, distribution, and bioavailability of carbamate and thiocarbamate pesticide residues in food and plant products for human consumption (e g , 3, 22, 23, 312, 346-348) A detailed discussion of the subject is out of the scope of this Section. The residue tolerance level permitted in harvested plant food is 7 mg/kg for Ziram, Thiram, and Ferbam whereas that of Maneb is 2-10 mg/kg (312) The World Health Organization (349) has provisionally recommended 0-0.005 mg/kg body weight as the "acceptable" daily intake A rebuttable presumption against the registration and continued registration of pesticide products containing Diallate has been forwarded by the U.S. Environmental Protection Agency, because of the increasing evidence of carcinogenicity and mutagenicity of the compound (161) Based on structural and metabolic consideration, the whole class of S-chloroallyl thiocarbamates may probably pose a potential carcinogenic risk to exposed humans As of 1973, the use of Monuron in agricultural crop protection is no longer registered (350), Monuron is now mainly used in non-crop applications, such as landscaping

SUPPLETORY NOTE FOR SECTION 5.2.1.6

The cell cycle of a growing cell is the interval from one cell division to the next. A series of highly programmed, time sequence, biochemical events that are common to almost all cell types are known to occur in the interphase, a period between cell division and the onset of the following mitosis. The terminology of the cell cycle was introduced in 1953 by Howard and Pelc (351) and has since been broadly adopted. This terminology formalizes the time sequence of cell division with respect to DNA synthesis. Studies using microspectrophotometry (352) or autoradiography (351) have demonstrated that DNA replication takes place only during a certain part of the interphase and not in mitosis. Using DNA synthesis as a marker, the interphase can be subdivided into G_1, S and G_2 periods; S denotes the period of DNA synthesis while G_1 (for Gap 1) and G_2 (for Gap 2) designate the pre and post intervals in the interphase during which no nuclear DNA is synthesized. Early stages of chromosome condensation, synthesis and assembly of the mitotic apparatus are presumed to occur in G_2. Following G_2 begins the period of cell division called the D phase (for division) or more frequently the M phase (for mitosis).

Owing to the improvement of cell culture techniques during the past decades, the duration of each period of the cell cycle can be determined. This can be accomplished by autoradiographic assay for isotope in the chromosomes of cells in mitosis at various time intervals after a single pulse exposure of the cells to

^{3}H-thymidine. Alternatively, it can also be determined by analyzing the cell growth curve following addition and subsequent removal of an inhibitor of DNA synthesis (e.g., 5-fluorodeoxyuridine) or of mitosis (e.g., colchicine). The biology of the cell cycle and detailed methods of cell cycle analysis have been described (353-357). In general, the duration of S, G_2 and M are relatively constant and quite similar in most mammalian cells: S about 6 to 9 hours, G_2 about 2 to 5 hours, and M 0.2 to 1.0 hour (356). Conversely, great variation occurs in the length of G_1, which may be unmeasurably short or may last days or even weeks depending on the cell type (354, 356). Accordingly, the length of the cell cycle of any cell type is largely affected by the length of G_1. Moreover, it has been shown that changes in generation time of cells in culture, due to alteration of culture conditions, also stem predominantly from prolongation or shortening of G_1 with little or no changes of S, G_2 or M (358).

While S and M are relatively well defined, information about the biochemical events that occur in G_1 and G_2, as well as their roles in the progress of the state of the cell within the cycle is still meager. However, once a cell enters S from G_1, it will automatically transit to G_2, undergo mitosis, and then divide. The initiation of DNA synthesis appears, therefore, to be a critical step in the regulation of cell proliferation. Studies with inhibitors have shown that both RNA synthesis and protein synthesis are required for the transition of G_1 to S. Based on the observations that new daughter cells with smaller mass

synthesize more protein during the G_1 period and also have a longer length of G_1 than those with larger mass, it was suggested that a certain quantity of protein must be synthesized before DNA replication can be initiated (359). In accord with these observations are the findings that if protein synthesis is blocked for a certain time during G_1, the transition to S is delayed for an equally long period (360). Little is known at present about the nature of proteins involved in the control of DNA replication in the eukaryotes. Enzymes mediating DNA synthesis such as DNA polymerase, thymidylate synthetase, kinase etc. have been shown to sharply increase in liver and kidney cells immediately before DNA replication (361, 362). However, these enzymes in other cell types were found present throughout the G_1 phase and have no significant increase with respect to the onset of DNA synthesis (363, 364).

Protein synthesis is required not only for the initiation but also for the successful completion of replication of DNA in eukaryotes (365, 366). Although the role of histone in the structure and function of the chromatin is not yet clearly understood, there is ample evidence that histone synthesis occurs concurrently with DNA synthesis (e.g., 367, 368). Moreover, the phosphorylation of histone H1 appears to be intimately involved in the modification of chromatin structure during the entire cell cycle (369, 370).

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NOTES ADDED AFTER COMPLETION OF SECTION 5.2.1.6

Hedenstedt et al. (1) tested the mutagenicity of 12 thiram and dithiocarbamate compounds in the Ames test. Thiram was found to be a much more potent mutagen than its monosulfide derivative (tetramethylthiram monosulfide), inducing reversions in the base-substitution- and possibly frameshift-sensitive strains. Disulfiram, the ethyl homolog of thiram, was inactive. Among the 9 dithiocarbamates tested, four (tellurium diethyl-, zinc dibutyl-, nickel dibutyl-, piperidinum pentamethylene-) are inactive. The mutagenic compounds (mostly towards base-substitution mutants with no requirement of metabolic activation) include, in this decreasing order of potency: zinc dimethyl- (ziram), cadmium diethyl-, zinc diethyl-, zinc ethylphenyl-, and copper dimethyldithiocarbamates. Comparison of the bacterial mutagenicity with in vitro alkylating activity [reaction with 4-(p-nitrobenzyl)-pyridine or deoxyguanosine] shows no correlation (2). Koga et al. (3) have demonstrated that, in contrast to the lack of mutagenicity of ethyl carbamate in the Ames test, alkyl N-hydroxycarbamates exhibited weak but significant mutagenic activity. The relative mutagenic potency of alkyl N-hydroxycarbamates follows the order: ethyl >> propyl > methyl. Acylation of ethyl N-hydroxycarbamate markedly enhances its mutagenic activity. Ethyl N-benzoyloxycarbamate, the most active compound, is about 260 times more potent than ethyl N-hydroxycarbamate (based on comparison of revertants/nmole using strain TA 100). Further N-acylation, yielding N-acyloxy-N-acylcarbamates does not bring about additional increases in mutagenicity. Another closely related compound, methyl N-methylolcarbamate (which is employed to obtain a flame-retardant finish on cotton) has been tested by MacGregor et al. (4) and found to be inactive in the Ames test. Douglas et al. (5) reported that Diallate and Triallate exhibited mutagenic activity towards both base-substitution and frameshift mutants.

In other mutagenicity tests, Zdzienicka et al. (6) found that thiram was positive in prophage induction, Salmonella typhimurium repair and Aspergillus nidulans forward-mutation tests. Benomyl was negative in gene-conversion tests using Saccharomyces cerevisiae or Aspergillus nidulans (7). Carbaryl was negative in the micronucleus test (8). Diallate and Triallate caused cnromosome damage or sister-chromatid exchanges (SCE) in cultured mammalian cells (5). Both vinyl carbamate and urethan (ethyl carbamate) induced SCE in cultured human lymphocytes; however, the activity of the former was enhanced while that of the latter was reduced by S-9 mix (9). Cheng et al. (10) compared the SCE-inducing ability of four alkyl carbamates, the relative potency: ethyl > ethyl N-hydroxy- > isopropyl > methyl (inactive) is in good agreement with their carcinogenic potency.

The carcinogenicity of vinyl carbamate has been further tested by Dahl et al. (11) and compared to urethan and several other carbamates. When administered during the first 3.5 or 5 weeks after birth, both vinyl carbamate and urethan induced liver tumors, thymomas, lung adenomas, and Harderian gland tumors in C57BL/6J x C3H/HeJ mice or ear duct and hepatic carcinomas and neurofibrosarcomas of the ear lobe in Fischer rats. Vinyl carbamate was significantly more active than urethan in the induction of various types of tumors. In mouse (strain A/J) lung adenoma assay, deuterated (CD_3CD_2-) urethan was as active as unsubstituted (CH_3CH_2-) urethan whereas t-butyl carbamate was inactive. The final analysis of NCI carcinogenicity data on Diallate (Avadex) and potassium bis-(2-hydroxyethyl)-dithiocarbamate has recently been completed (12). In contrast to some previous reports (see Section 5.2.1.6.3), it appears that both compounds do not have statistically significant carcinogenic effects in Charles River CD rats.

A variety of agents have been tested as potential modifiers of carcinogenesis by carbamate compounds. High doses of the mixed-function oxidase inhibitor 2-(2,4-dichloro-6-phenyl)-phenoxyethylamine inhibited the induction of lung adenomas by ethyl N-hydroxycarbamae but had no effect on vinyl carbamate or urethan. On the other hand, caffeine inhibited the lung adenoma induction by urethan, but had no consistent effect on vinyl carbamate or ethyl N-hydroxycarbamate (11). The lung adenoma assay has also been utilized by Theiss et al. (13) who investigated the potential of commercial saccharin as a promotor or co-carcinogen of urethan. At the low (0.1 g/kg) dose of urethan, saccharin had no effect. At the high (1 g/kg) dose, however, saccharin significantly increased the number of adenomas per mouse over the number found in mice treated with urethan alone. The carcinogenic effect of combined treatment of CFLP mice with urethan and diethylstilbestrol (DES) was studied by Ferenc et al. (14). The incidence of lymphoma was 0% in controls, 0% with urethan only, 4.1% with DES only, 18.1% with DES followed by urethan 14 days later, 32% with urethan and DES given simultaneously, and 44% with urethan followed by DES 14 days later. All urethan-treated mice developed lung tumors regardless of DES treatment. In a preliminary communication, Witschi (15) reported that butylated hydroxytoluene (BHT) enhanced the lung tumor-inducing effect of urethan in Swiss-Webster mice if administered after urethan. Viral infection has been shown to suppress the pulmonary adenoma-inducing effect of urethan in mice, a brief review of this topic has been presented by Nettesheim et al. (16). The development of lung tumors was observed in Kid:CFLP mice treated intragastrically with diethyl pyrocarbonate (an antimicrobial agent used as a perservative of beverages and food) and ammonia. The authors (17) suggested that the carcinogenic effect may result from the in vivo formation of urethan from the two precursors.

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