"Current Awareness" Program
Vol. VI #6

Halogenated Phenoxy Acids, Aromatic Ethers, Dibenzofurans Dibenzo-p-Dioxins

HALOGENATED PHENOXY ACIDS, AROMATIC ETHERS, DIBENZOFURANS AND DIBENZO-p-DIOXINS

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

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Preparation for the Chemical Hazard Identification Branch "Current Awareness" Program

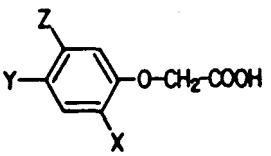
5.2.2.3 Halogenated Phenoxy Acids, Aromatic Ethers, Dibenzofurans and Dibenzo-p-dioxins.

5.2.2.3.1 Introduction.

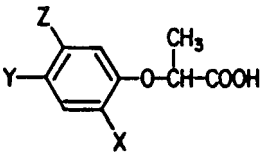
Halogenated phenoxy acids, aromatic ethers, dibenzofurans and dibenzo-p-dioxins, the structures of which are depicted in Table XLVI, include a number of controversial and notorious chemicals. Halogenated dibenzofurans and dibenzo-p-dioxins often occur as unwanted contaminants of polyhalogenated biphenyls, halogenated aromatic ethers and phenoxy acids (see Section 5.2.2.3.5.2).

Chlorophenoxy acids were first developed in the early 1940's as plant growth regulators shortly after the well known discovery, by Kögl and his associates, of indole-3-acetic acid (also known as IAA or auxin) as a natural plant growth hormone. They came into use as herbicides during World War II when the optimization of food production with a reduced labor force was a vital factor in the war effort. 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) are two of the most widely used herbicides for the control of broad-leaf weeds in many industrialized countries (1-4). The production of 2,4-D in the United States increased from 450 metric tons in 1945 to a maximum of 36,000 metric tons in 1968; production fell to 20,000 metric tons in 1970 but gradually increased again to an estimated 30,000 metric tons in 1977 (4). 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), an effective herbicide against woody and herbaceous weeds, was first produced commercially in the United States in 1944. The production of 2,4,5-T increased sharply between 1960 and 1968 (2, 5) when a 1:1 mixture of n-butyl esters of 2,4-D and 2,4,5-T was used as a defoliant in South Vietnam under the names of "Agent Orange," "Herbicide Agent Orange," or "Herbicide

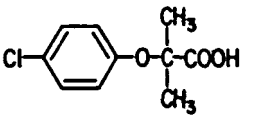
TABLE XLVI



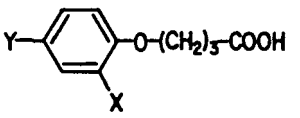
2,4-D(X=Cl; Y=Cl; Z=H)
MCPA(X=CH_3; Y=Cl; Z=H)
2,4,5-T(X=Cl; Y=Cl; Z=Cl)



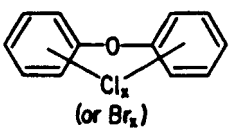
Dichloroprop(X=Cl; Y=Cl; Z=H)
Mecoprop(X=CH_3; Y=Cl; Z=H)
Silvex(X=Cl; Y=Cl; Z=Cl)



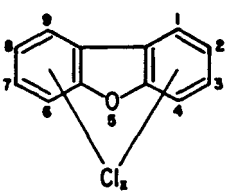
Clofibrate



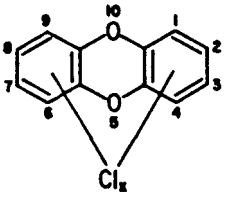
2,4-DB(X=Cl; Y=Cl)
MCPB(X=CH_3; Y=Cl)



Halogenated Diphenyl Ethers



Chlorinated Dibenzofuran



Chlorinated Dibenzo-p-dioxin

Orange." The amount of Agent Orange sprayed during the Vietnam war was estimated to exceed 40 metric tons (Whiteside, cited in ref. 5). Concern over the potential long-term health hazards of chlorophenoxy acids first arose becasue of a study by the Bionetics Research Laboratory (6) indicating teratogenic effects of technical-grade 2,4,5-T in rodents. Since 1969, the U.S. Government has restricted or suspended the use of 2,4,5-T in populated areas. Although the teratogenicity of technical-grade 2,4,5-T was later attributed mainly to the action of an extremely potent teratogenic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), there is some evidence that some relatively uncontaminated chlorophenoxy acids may be teratogenic (albeit weakly so) in certain animal species (see Section 5.2.2.3.2.2). Recent epidemiologic studies by Hardell and his associates (7, 8; see also Section 5.2.2.3.5.2) suggest that human exposure to chlorophenoxy acids (including 2,4-D and MCPA, which are usually not contaminated with TCDD) may represent an increased carcinogenic risk for several types of tumors. These results, coupled with the known carcinogenicity of clofibrate (see Table XLVI; see also Section 5.2.1.7 in Volume IIIA) indicate that apart from possible contamination with TCDD, chlorophenoxy acids, as a class, may pose long-term health hazard to humans and should be more thoroughly investigated.

Chlorinated dibenzo-p-dioxins have long been recognized as possible byproducts in the production of certain chlorinated phenols. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), an extremely toxic compound, is known to be formed in the manufacturing of products involving 2,4,5-trichlorophenol as an intermediate. Since 1949, over 200 TCDD-related industrial accidents have occurred around the world (9). The most characteristic and frequently observed toxic effect in humans is chloracne, a severe form of dermatitis. Suspicion of the possible long-term health hazards of TCDD arose when it was

found in the Bionetics Research Laboratory study (6) that 2,4,5-T is teratogenic in the rat and the mouse. In fact, later tests indicated that these teratogenic effects may have been caused by trace amounts (27 ± 8 ppm) of TCDD present as a contaminant in the 2,4,5-T sample used in the Bionetics study. The possibility that TCDD may be carcinogenic in humans was raised by Tung (10) who reported an increase in the incidence of liver cancers among Vietnamese during 1962-1968 when TCDD-contaminated defoliant, Agent Orange, was sprayed in the countryside. Several well publicized incidents of public exposure to TCDD -- the accidental release of a massive amount of TCDD into the atmosphere in Seveso (Italy) in 1976; the finding of TCDD in Love Canal, Niagara Falls; the spraying of TCDD-contaminated waste oil for dust control in Missouri (see Section 5.2.2.3.5.2) -- further stimulated an explosive growth in the investigations of the health hazards and environmental significance of TCDD and related compounds. The deep concern over, and intense interest in, TCDD and related compounds is reflected by the large number of review articles, monographs and symposia (11-23).

5.2.2.3.2 Physicochemical Properties and Biological Effects.

5.2.2.3.2.1 PHYSICAL AND CHEMICAL PROPERTIES.

The physical and chemical properties of chlorophenoxy acids have been described in detail by Melnikov (1). Some physicochemical properties of these compounds are summarized in Table XLVII. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a relatively strong acid with a pKa of 2.64. It reacts with organic and inorganic bases to form stable salts (which are substantially more soluble in water) and with alcohols to form esters. The lower alkyl esters of 2,4-D are relatively volatile (e.g., 2,4-D isopropyl ester has a vapor pressure of 1.05×10^{-2} mm Hg at 25°C); the volatility decreases with an increase

Table XLVII
Physicochemical Properties of Some Chlorinated Phenoxy Acids and Dibenzodioxins[a]

Compound	M.W.	m.p.(°C)	pK_a	Solubility
2,4-Dichlorophenoxy-acetic acid (2,4-D)	221.0	141	2.64	Water, 0.54 g/l (20°C); ethanol, 1.3 kg/kg; ether, 2.43 kg/kg; toluene, 0.67g/kg; soluble in most organic solvents
2-Methyl-4-chlorophenoxy-acetic acid (MCPA)	200.6	120	3.27	Water, 0.63 g/l (20°C); highly soluble in alcohol, ether, carbon tetrachloride, benzene and organic solvents
2,4,5-Trichlorophenoxy-acetic acid (2,4,5-T)	255.5	158-159		Water, 0.189 g/l (20°C); highly soluble in alcohol, ether, chloroform and benzene
2-(2,4-Dichlorophenoxy)-propionic acid (Dichloroprop)	235.1	117-118		Water, 0.35 g/l (20°C); soluble in most organic solvents
2-(2-Methyl-4-chlorophenoxy)-propionic acid (Mecoprop)	214.6	94-95		Water, 0.62 g/l (20°C)
2-(2,4,5-Trichlorophenoxy)-propionic acid (Silvex)	269.5	179-181	2.84	Water, 0.14 g/l (25°C); acetone, 180 g/kg; methanol, 134 g/kg; ether, 98 g/kg; heptane, 0.86 g/kg
4-(2,4-Dichlorophenoxy)-butyric acid (2,4-DB)	249.1	117-119	4.8	Water, 0.053 g/l; acetone, 100 g/kg
4-(2-Methyl-4-chlorophenoxy)-butyric acid (MCPB)	228.6	100-101		Water, 0.044 g/l; ethanol, 150 g/kg; acetone, 200 g/kg
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	322	305		Water, 0.2ug/l (0.2 ppb); *o*-dichlorobenzene, 1.4 g/l; benzene, 0.57 g/l; chloroform, 0.37 g/l; n-octanol, 0.048 g/l; acetone, 0.11 g/l
Octachlorodibenzo-*p*-dioxin (OCDD)	459.8	130		*o*-Dichlorobenzene, 1.83 g/l; xylene, 3.58 g/l; chloroform, 0.56 g/l; dioxane, 0.38 g/l

[a]Summarized from data compiled by N.N. Melnikov [Residue Rev. 36, 157 (1971)]; C.R. Worthington (ed.): "The Pesticide Manual," 6th ed., British Crop Protection Council, 1979; M.P. Esposito, H.M. Drake, J.A. Smith, and T.W. Ownes: "Dioxins: Volume I. Sources, Exposure, Transport, and Control," EPA-600/2-80-156, U.S. Environmental Protection Agency, Washington, D.C., 1980.

molecular weight. Similar properties have been found with other chlorophenoxy acids. Chlorophenoxy acids are relatively susceptible to breakdown by enzymes in plant tissues (e.g., 24) or by microorganisms in the soil (25).

The physical and chemical properties of halogenated dibenzofurans and dibenzo-p-dioxins have not been thoroughly studied due to the extreme toxicity of some members of these two classes of compounds. Theoretically, depending on the number of chlorine atoms and the position(s) of substitution, there are 75 possible congeners or isomers of chlorinated dibenzo-p-dioxins (2 mono-, 10 di-, 14 tri-, 22 tetra-, 14 penta-, 10 hexa-, 2 hepta-, and 1 octa-chloro-isomers) and 135 chlorinated dibenzofurans (4 mono-, 16 di-, 28 tri-, 38 tetra-, 28 penta-, 16 hexa-, 4 hepta-, and 1 octa-chloro-isomers) (17). Some 40 of the 75 possible chlorinated dibenzo-p-dioxins have been prepared and identified (see ref. 22). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the prototype compound of the chlorinated dibenzo-p-dioxins. It is remarkable for its lack of reactive functional groups and its chemical stability (13). In fact, it is thermally stable up to the high temperature of 700°C. Complete decomposition occurs at 800°C (26). The half-life of TCDD in the soil has recently been estimated to exceed 10 years (27). It is an extremely lipophilic compound, practically insoluble in water (0.2 ppb) and only sparingly soluble in most organic solvents (see Table XLVII). Both chlorinated dibenzo-p-dioxins (22, 26) and dibenzofurans (28) are, however, subject to photochemical degradation with dechlorination as the principal reaction. The photolytic susceptibility of three chlorinated dibenzo-p-dioxins follows the order: 2,7-dichloro- $\geq$ 2,3,7,8-tetrachloro- > octachloro- (26). A synoptic review of photolytic studies on TCDD has been presented by Esposito et al. (22).

5.2.2.3.2.2 BIOLOGICAL EFFECTS OTHER THAN CARCINOGENICITY.

Toxic and Other Biochemical Effects. The toxicology of halogenated phenoxy acids has been reviewed in several publications (2, 29, 30). Table XLVIII summarizes some representative acute toxicity data of 2,4-D, 2,4,5-T and several related compounds in three mammalian species. In general, uncontaminated halogenated phenoxy acids are only moderately toxic to mammals probably because of their rapid renal excretion (see Section 5.2.2.3.4). Acute toxic effects in mammals include various signs of muscular disorders causing stiffness of extremities, ataxia, paralysis and eventually coma (31), disruption of T-tubules in the myocardium, hypocholesterolemia and reduction of serum triglyceride (30). Workers involved in the production of 2,4,5-T were reported to develop chloracne, liver disorders, neurologic changes, metabolic disorders and porphyria (32); these effects appear to be due primarily to the action of the extremely toxic contaminant, TCDD (see below).

The structure-activity relationships of chlorophenoxy acids as herbicides have been extensively studied (reviewed in refs. 1, 3). Chlorophenoxy acids are structurally similar to the natural plant growth hormone, indole-3-acetic acid (also known as auxin or IAA) and exert their herbicidal action by causing lethally abnormal growth. Some of the important structural features of herbicidal chlorophenoxy acids are: (a) the side-chain must possess a terminal carboxyl group or a group that is easily converted to it within the plant tissues; (b) the side-chain must contain an odd number of methylene groups and at least one hydrogen atom attached to the α-carbon; chlorophenoxy acids with 3 or higher odd number of methylene groups owe their herbicidal activity to in vivo β-oxidation to active chlorophenoxyacetic acid derivatives; (c) among the chlorophenoxy acids with asymmetric carbon (e.g., dichloroprop, mecoprop,

Table XLVIII
Acute Oral Toxicity of 2,4-D, 2,4,5-T and Related Compounds in Mammalian Species[a]

Compound	LD_{50} (mg/kg)		
	Mouse	Rat	Guinea pig
2,4-Dichlorophenoxyacetic acid (2,4-D)[a]			
acid	368 (M)	375 (M)	469
sodium salt	375	805 (F)	551 (M)
isopropyl ester	541 (M)	700	550 (M)
mixed butyl esters	713 (F)	620 (F)	848 (F)
2-Methyl-4-chlorophenoxyacetic acid (MCPA)[b]			
acid	550	700	
diethanolamine salt	550	800	
2-(2,4-Dichlorophenoxy)propionic acid (Dichloroprop; 2,4-DP)[b]			
acid	400	800	
2-(2-Methyl-4-chlorophenoxy)propionic acid (Mecoprop)[b]			
acid	650	700-1,500	
diethanolamine salt	600	1,060	
4-(2,4-Dichlorophenoxy)butyric acid (2,4-DB)[b]			
acid		370-700	
sodium salt	400	700-1,500	
4-(2-Methyl-4-chlorophenoxy)butyric acid (MCPB)[a]			
acid		680	
sodium salt	700		
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)[a]			
acid	389 (M)	500 (M)	381
isopropyl ester	551 (F)	495	449 (F)
mixed butyl ester	940 (F)	481 (F)	750 (F)
mixed amyl ester		750 (F)	

[a]Summarized from the data of V.K. Rowe and T.A. Hymas [Am. J. Vet. Res. 15, 622 (1954)].

[b]Summarized from the data compiled by J.P. Seiler [Mutat. Res. 55, 197 (1978)].

silvex), only the (+)-forms of the stereoisomers are active; and (d) the introduction of chlorine atoms into

The 2-, 4-, or 2,4-positions of the phenyl ring greatly enhances activity whereas poor activity is found for compounds containing chlorine atoms at the 2,6- or 3,5-positions. It has been hypothesized (Thimann's theory) that the activity of IAA, chlorophenoxy acids, and other herbicides such as 2,3,6-TBA (2,3,6-trichlorobenzoic acid) and picloram (2-carboxy-4-amino-3,5,6-trichloropyridine) is dependent on the presence of a fractional positive charge (e.g., at the 1-position of IAA, 6-position of 2,4-D, 4-position of 2,3,6-TBA, 4-amino group of picloram) situated at a 5.5 Å distance from the negative charge of the carboxyl group. Presumably this spacing of the polar centers is a requirement for the binding of these compounds to receptor sites in plant cells so as to exert a phytohormonal action (see ref. 3).

The toxicology of halogenated dibenzofurans and dibenzo-p-dioxins has been a subject of extensive studies in recent years because of their environmental significance and the extreme toxicity of some members of these two classes of compounds. Numerous review articles, monographs and symposia (11, 12, 16-21, 30, 33) have covered this topic; only a brief account emphasizing the comparative toxicity and structure-activity relationship is presented below. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the most extensively studied compound of the chlorinated dibenzodioxin series, has been widely regarded as probably the most highly toxic compound synthesized so far. It produces a gamut of toxic effects in a variety of animal species. Wide species variations in lethality and pathologic responses have been observed. The acute oral LD_{50} values (in μg/kg body weight) of TCDD in mammalian species are: 0.6-2 for guinea pigs (34, 35) 22-60 for rats (34, 36); < 70 for monkeys

(37), 115 for rabbits (34), 114-284 for mice (35, 38, 39), > 300 for dogs (34), and > 3,000 for hamsters (40). The unusual resistance of hamsters to TCDD toxicity may be partially, but not fully, explained by a much faster rate of metabolism and elimination of the compound in this species (41). The acute toxicity of TCDD in rats is decreased by pretreatment of the animals with inducers of microsomal mixed-function oxidases (MFOs), but enhanced by MFO inhibitors, suggesting that the metabolism of TCDD is mainly detoxifying in nature (36). Most of the pathologic responses to TCDD involve epithelial tissues. The types of toxic responses include (a) hyperplasia and/or metaplasia of the skin (chloracne), gastro-intestinal mucosa, urinary tract, bile duct and/or gall bladder, (b) hypoplasia, atrophy or necrosis of thymus, bone marrow and testicles, (c) hepatomegaly or liver necrosis, (d) edema, and (e) wasting (severe weight loss) accompanied by a depletion of adipose tissue; many of these responses are highly species-specific (see refs. 18, 20). A number of isosteric analogs of TCDD, such as 2,3,7,8-tetrachlorodibenzofuran, 2,3,6,7-tetrachloronaphthalene, and 3,3',4,4'-tetrachlorobiphenyl, appear to have a similar spectrum of toxic effects as TCDD (20).

The structure-toxicity relationships of halogenated dibenzo-p-dioxins, dibenzofurans and related compounds have been extensively investigated by McConnell, Moore and associates (18, 35, 37, 42). The acute, oral LD_{50} values of 13 chlorinated dibenzo-p-dioxins and several related compounds in mice and guinea pigs are summarized in Table XLIX. As the data in the Table indicate, the degree of toxicity of chlorinated dibenzo-p-dioxin is dependent upon the number and positions of chlorine substitutions. The most toxic compound (TCDD) is at least 180,000 times more potent than the least toxic compound (2,8-dichlorodibenzo-p-dioxin). It is apparent that all the lateral positions (2,3,7,8-) must be chlorinated to achieve the greatest degree of toxicity.

Table XLIX
Estimated Single Oral 30-Day LD_{50} Values of Chlorinated Dibenzo-p-dioxins and Related Compounds[a]

Compound	LD_{50} (umole/kg)		Relative Potency in the Guinea Pig[b]
	Mouse	Guinea pig	
Substituted Dibenzo-p-dioxin			
2,8-Dichloro-	—	> 1,180	0.01
2,3,7-Trichloro-	> 10	120.4	0.09
2,3,7,8-Tetrachloro-	0.88	0.006	1,883
1,2,3,7,8-Pentachloro-	0.94	0.009	1,256
1,2,4,7,8-Pentachloro-	> 14	3.15	3.6
1,2,3,4,7,8-Hexachloro-	2.11	0.185	61
1,2,3,6,7,8-Hexachloro-	3.19	0.178-0.255	44-63
1,2,3,7,8,9-Hexachloro-	> 3.67	0.153-0.255	44-74
1,2,3,4,6,7,8-Heptachloro-	—	> 1.4	8
1-Nitro-3,7,8-trichloro-	—	> 90	0.13
1-Amino-3,7,8-trichloro-	—	> 99	0.11
1-Nitro-2,3,7,8-tetrachloro-	> 5.4	0.129	88
1-Amino-2,3,7,8-tetrachloro-	> 14.2	0.576	20
Most Toxic Congener of Related Halogenated Aromatic Series			
2,3,7,8-Tetrachlorodibenzofuran	—	0.023	507
3,3',4,4',5,5'-Hexachlorobiphenyl	—	1.39	8
2,3,6,7-Tetrachloronaphthalene	—	> 11.3	1
2,3,6,7-Tetrabromonaphthalene	—	0.547	21

[a]Summarized from E.E. McConnell, J.A. Moore, J.K. Haseman, and M.W. Harris [Toxicol. Appl. Pharmacol. 44, 335 (1978)] and E.E. McConnell: In "Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products" (R.D. Kimbrough, ed.), Elsevier, Holland, 1980, p. 109.

[b]Relative to 2,3,6,7-tetrachloronaphthalene.

Additional chlorine atoms (or amino or nitro groups) at ortho (or peri) positions reduce the toxicity but not to the extent caused by deletion or substitution of a chlorine atom at one of the lateral positions. Interestingly, the same structural requirements for toxicity are also observed in the halogenated dibenzofuran, biphenyl and naphthalene series (see Table XLIX). The relative toxicity of the most toxic chlorinated congener of each of the above series follows the order: dibenzo-p-dioxin > dibenzofuran >> biphenyl >> naphthalene. Considering the isosteric nature (a rectangular molecular shape of about 6 x 11 Å* with a halogen at each of the 4 lateral positions) of all these compounds, the similarity of toxic effects, and other inferential evidence, Poland and Knutson (20) proposed that they have a common mechanism responsible for the toxicity. According to Poland and Knutson, these compounds bind to a common cytosolic receptor and the receptor-ligand complex translocates to the nucleus and mediates the ensuing gene expression causing two stages of pleiotropic responses -- a "limited" response involving a variety of biochemical effects (see below) and a "restricted" response involving the expression of normally restricted genes thus leading to a gamut of toxic effects. In this connection the reader is cautioned that other isosteric compounds (such as dibenzothiophene, thianthrene, thioxanthone, phenothiazine, anthraquinone) with lateral positions fully halogenated may be potentially toxic.

Other biochemical effects, produced by TCDD and its isosteric analogs, and the structure-activity relationships have been reviewed in detail by Poland and associates (13, 20) and by Goldstein (43). Essentially, these effects are: (a) endocrine effects (abnormal levels of estrogens, thyroxine and corticosteroids); (b) vitamin A deficiency; (c) abnormal lipid metabolism (causing fatty liver and possible increase in lipid peroxidation); (d) porphyria; (e) impaired biliary and renal excretory transport; and (f) induction

* These figures represent the approximate molecular size of biphenyl and dibenzo-p-dioxin calculated with inclusion of the van der Waal radii.

of microsomal mixed-function oxidases (MFOs). Among these, the induction of MFOs by TCDD and congeners has been extensively studied (this topic will be further discussed in Section 9.4 in a subsequent volume of this series). TCDD is generally considered to be probably the most potent inducer of MFOs; on a molar basis, TCDD (median effective dose, ED_{50} = 0.85 nmol/kg) is 30,000 times more potent that 3-methylcholanthrene. The inducing effect of a single dose of TCDD may persist for 35 days in rats, reflecting the prolonged biological half-life of the compound. The inducing effect of TCDD and congeners has been shown to be mediated through a cytosolic receptor protein which binds the ligand, translocates to the DNA and initiates enzyme induction. Structure-activity relationship studies show a good correlation between their binding affinities to the receptor protein and their potency to induce aryl hydrocarbon hydroxylase (AHH) activity. The structural requirements for enzyme induction are similar to those described for toxicity.

Mutagenicity. Owing to their environmental importance, halogenated phenoxyacetic acids, dibenzodioxins and related compounds have been extensively tested for mutagenicity. Table L summarizes the data available on Ames Salmonella tests of a variety of halogenated phenoxyacetic acid, diphenyl ether, dibenzofuran, and dibenzo-p-dioxin derivatives. As the data in the Table indicate, some 20 derivatives of 2,4-D and 2,4,5-T have been tested; interestingly, none of these compounds show any mutagenic activity.

Based on structural similarity to acridine compounds (see Section 5.1.2.2.2, Vol. IIB), TCDD is expected to be a frame-shift mutagen (15). The evidence for mutagenicity of TCDD is, however, unconvincing with the Ames test. Using a liquid preincubation procedure, Hussain et al. (59) reported that TCDD was mutagenic without metabolic activation in Salmonella typhimurium strain TA1532 (a frame-shift mutant) but inactive in strain TA1530 (a base-

Table L
Mutagenicity of 2,4-D, 2,4,5-T, TCDD and Related Compounds in the Ames Test

Compound	Mutagenicity	References
2,4-D, 2,4,5-T and Related Compounds		
4-Chlorophenoxyacetic acid	-	(44*)
2,4-Dichlorophenoxyacetic acid (2,4-D)		
acid or sodium salt	-	(44*, 45-48)
n-butyl ester	-	(49)
isooctyl ester	-	(50)
3,4-Dichlorophenoxyacetic acid	-	(44*)
2-Methyl-4-chlorphenoxyacetic acid (MCPA)	-	(51)
2-(4-Chlorophenoxy)propionic acid	-	(44*)
2-(2,4-Dichlorophenoxy)propionic acid (Dichloroprop)	-	(44*)
2-(2-Methyl-4-chlorophenoxy)pripionic acid (Mecoprop)	-	(44*, 52)
4-Chlorophenoxyisobutyric acid (Clofibrate)	-	(53)
2-(2,4-Dichlorophenoxy)butyric acid (2,4-DB)	-	(44*, 45)
4-(3,4-Dichlorophenoxy)butyric acid	-	(44*)
4-(2-Methyl-4-chlorophenoxy)butyric acid (MCPB)	-	(44*)
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)		
acid	-	(44*, 54, 55)
n-butyl ester	-	(49, 54)
isobutyl ester	-	(49)
2-(2,4,5-Trichlorophenoxy)propionic acid (Silvex)	-	(44*)
4-(2,4,5-Trichlorophenoxy)butyric acid	-	(44*)
Halogenated Diphenyl Ether		
Decabromodiphenyl ester	-	(56)
Chlorinated Dibenzofurans		
Unsubstituted dibenzofuran	-	(57, 58)
2,9-Dichlorodibenzofuran	-	(57)
3,6-Dichlorodibenzofuran	-	(57)
2,3,7,8-Tetrachlorodibenzofuran	-	(57)
Octachlorodibenzofuran	-	(57)
Chlorinated dibenzo-p-dioxins		
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	+/?	(59, 60)
	-	(56, 61, 62)
Octachlorodibenzo-p-dioxin	?/-	(60)

*Without metabolic activation

pair substitution mutant). Seiler (60) confirmed the mutagenicity of TCDD in TA1532 in a spot test but showed marginal or a lack of activity in two other frame-shift mutants (TA1531, TA1534) and two base-pair substitution mutants (G46, TA1530). Unpublished results of McCann (cited in ref. 15), however, failed to show any mutagenic activity of TCDD in strains TA1532, TA1535, TA1537 and TA1538 with or without metabolic activation in a plate incorporation test (which is more sensitive than the spot test). Negative findings have also been reported by Nebert et al. (61), Geiger and Neal (62), and by the U.S. National Toxicology Program (56) using strains TA98, TA100, TA1535, TA1537 and/or TA1538. Strain TA1537 is a more sensitive direct descendant of TA1532. Strain TA1538 and its plasmid-containing descendant, TA98, are also highly sensitive to frame-shift mutagens. The assay by the U.S. National Toxicology Program (56) used a liquid preincubation procedure, similar to that used by Hussain et al. (59) for obtaining their positive finding. The discrepancy betweem earlier and more recent studies may be due to differences in solvent (dimethylsulfoxide in earlier studies; 1,4-dioxane in recent studies), impurities or other factors. Nonetheless, the totality of the data tends to support the view that, like most polyhalogenated aromatics (see Section 5.2.2.2.2), TCDD has no appreciable mutagenic activity. It should be noted that, because of its extremely high toxicity, TCDD can only be assayed at microgram levels. It would be interesting to test congeners of TCDD with lower toxicity. Thus far, the considerably less toxic octachlorodibenzo-p-dioxin has been tested and found to have questionable or no activity (60). In the closely related dibenzofuran series, none of 4 chlorinated derivatives exhibited any mutagenic activity (see Table L).

In addition to the Ames Salmonella test, 2,4-D (reviewed in ref. 4), 2,4,5-T (reviewed in ref. 5), TCDD (reviewed in refs. 15, 19, 21) and related

compounds have been tested in a variety of test organisms including prophages (59), other bacteria (e.g., ref. 45; see also refs. 4, 5), Drosophila (63; see also refs. 4, 5), higher plants (64, 65; see also refs. 4, 5), cultured mammalian cells (45, 46, 66, 67) and experimental animals (54, 55, 68-71). With a few exceptions, these compounds are generally inactive or marginally active in most microbial and mammalian assays, but may display some activity in certain plants. It should be noted that the mutagenicity of halogenated phenoxyacetic acids in in vitro systems may be affected by the pH of the incubation medium, because of the inability of anionic forms to penetrate the cell membrane (48); also, studies with plants are often difficult to perform because of the auxin-like action of these compounds (see ref. 4). Cytogenetic studies have been conducted on the TCDD-exposed population in Seveso, Italy; thus far, the rate of chromosome aberrations appears to lie within the normal range (23, 72).

Teratogenicity.

The teratogenicity of 2,4,5-T (technical grade) was first reported in a 1968 teratogen screening study conducted by the Bionetics Research Laboratory (6) for U.S. National Cancer Institute. Subsequently, it was found that the teratogenic effects observed were attributable, at least in part, if not wholly, to the TCDD contaminant (30 ppm) present in the 2,4,5-T samples. Further studies confirmed the teratogenicity of TCDD and established it as one of the most potent animal teratogens known. Owing to their environmental significance, the teratogenicity of 2,4-D, 2,4,5-T, TCDD and related compounds has been extensively studied. A summary of the major findings of the teratogenicity studies on these compounds is presented in Table LI.

2,4-Dichlorophenoxyacetic acid (2,4-D) and several and its alkyl esters

Table LI
Teratogenicity of 2,4-D, 2,4,5-T, TCDD and Related Compounds

Compound	Species	Teratogenicity[a] (effects)[b]	References
2,4-Dichlorophenoxyacetic acid (2,4-D) and Related Compounds			
2,4-D	Mouse	±	(6)
	Rat	-	(73)
		+ (SM)	(74)
	Hamster	± (SM)	(75)
2,4-D methyl ester	Mouse	-	(6)
2,4-D ethyl ester	Mouse	-	(6)
2,4-D butyl ester	Mouse	-	(6)
	Rat	+ (SM)	(74)
2,4-D isooctyl ester	Mouse	±	(6)
	Rat	+ (SM)	(74)
	-	-	(76)
2,4-D dimethylamine salt	Rat	+ (SM)	(74)
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) and Related Compounds			
2,4,5-T	Mouse	+[c] (CP, KA)	(6, 77, 78)
		+ (CP, KA)	(78)
		+ (CP)	(79, 80)
		+ (CP, SM)	(81, 82)
		± (CP)	(83)
	Rat	+[c] (KA, VA)	(6, 77)
		-	(78, 84-87)
	Rabbit	-	(87)
	Hamster	+[c] (SM, EA)	(75)
		-	(88)
	Sheep	-	(89)
	Monkey	-	(90)
2,4,5-T butyl ester	Mouse	+ (CP)	(85)
2,4,5-T isooctyl ester	Mouse	+ (CP)	(85)

Table LI (continued)

Compound	Species	Teratogenicity[a] (effects)[b]	References
2,4,5-T propylene glycol butyl ether ester	Mouse	+ (CP)	(85)
2,4,6-Trichlorophenoxy-acetic acid	Mouse	-	(6)
Silvex	Mouse	-	(85)
	Rat	-	(85)
Phenoxyacetic acid	Mouse	-	(82)
2,4,5-Trichlorophenol	Mouse	-	(82)
Chlorinated Dibenzo-p-dioxins			
2-Chloro-	Rat	-	(68)
2,3-Dichloro-	Rat	-	(68)
2,7-Dichloro-	Rat	-	(34, 68)
1,2,3,4-Tetrachloro-	Rat	-	(68)
2,3,7,8-Tetrachloro-	Mouse	+ (CP, KA)	(78, 91, 92)
		+ (CP)	(79)
	Rat	+ (KA, VA)	(78)
		± (VA)	(68, 93)
	Rabbit	+ (KA)	(94)
	Monkey	+ (SPA)	(95)
Hexachloro- (mixed isomers)	Rat	+ (CP, SM)	(34)
Octachloro-	Rat	-	(34)

[a]Symbols for teratogenicity: + = positive; - = negative; ± = inconclusive, marginal effect or may be considered as manifestation of embryotoxicity rather than teratogenicity.

[b]Abbreviations used: CP = cleft palate; SM = skeletal malformations; KA = kidney abnormalities; VA = visceral anomalies; EA = eye abnormalities; SPA = soft palate abnormalities.

[c]Teratogenicity attributable, at least in part, to the presence of TCDD as contaminant.

were tested for teratogenicity in several strains of mice in the screening study of Bionetics Research Laboratory (6). Among these, 2,4-D and its isooctyl ester were found to have possibly weak teratogenic effects in some strains of mice; however, the results were considered to have marginal significance, requiring further studies for confirmation. The butyl ester of 2,4-D was listed as fetotoxic, but probably not teratogenic, while the methyl and ethyl esters of 2,4-D exhibited no fetotoxic or teratogenic effects under the conditions of the study. Two other related compounds, 2,4-D isopropyl alcohol and 2-(2,5-dichlorophenoxy)propionic acid, gave conflicting results from which no conclusions could be drawn. In a three-generation study using Osborne-Mendel rats, Hansen et al. (73) found no evidence in support of the teratogenicity of 2,4-D. However, Khera and McKinley (74) reported that 2,4-D and its butyl, isooctyl, butoxyethanol and dimethylamine derivatives (not believed to contain TCDD contaminant) all induced fetopathy and increased incidence of skeletal anomalies following daily oral administration of 100-150 mg/kg on days 6-15 of gestation. In a more recent study by Unger et al. (76), no evidence of teratogenicity was noted in CD rats that received daily oral doses of 2,4-D isooctyl ester equivalent to up to 87.5 mg/kg 2,4-D during days 6-15 of gestation. Low incidence of fetal anomalies was observed in the offspring of Syrian golden hamsters receiving over 60 mg/kg 2,4-D during days 6-10 of gestation; the effect was not clearly dose-dependent (75).

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) has been tested for teratogenicity in six mammalian species. It is important to note that the purity of 2,4,5-T samples used in these studies plays a significant role in determining the teratogenicity of the compound; technical grade 2,4,5-T may contain sufficient amounts of TCDD contaminant (which is extremely teratogenic) to account for a part of or all the teratogenic effects observed. Technical grade

2,4,5-T was found to be teratogenic in several strains of mice inducing mainly cleft palate and kidney abnormalities (6, 77, 78). Similar teratogenic effects have been observed in a number of studies (78-82) using analytical grade 2,4,5-T (containing less than 1 ppm TCDD) indicating that 2,4,5-T per se is indeed teratogenic in mice. In the rat, however, only technical grade 2,4,5-T containing relatively high amounts of TCDD (30 ppm) was found to be teratogenic (6, 77); a variety of studies (78, 84-87) using more purified 2,4,5-T samples showed a complete lack of teratogenicity. Similarly, 2,4,5-T appeared to be nonteratogenic in rabbits (87), sheep (89) and monkeys (90). Collins and Williams (75) reported that commercial samples of 2,4,5-T induced eye abnormalities (absence of eyelid) and delayed head ossification in the fetuses of Syrian golden hamsters; the effects were related to the amount of TCDD contaminant present. In another hamster study, 2,4,5-T was not found teratogenic (88). Besides 2,4,5-T, the butyl, isooctyl and propylene glycol butyl ether esters of 2,4,5-T are all teratogenic in the mouse inducing mainly cleft palate (85). In contrast, a number of closely related compounds, such as 2,4,6-trichlorophenoxyacetic acid, silvex, phenoxyacetic acid and 2,4,5-trichlorophenol, are all inactive in the mouse suggesting that strict structural requirements are needed for teratogenicity.

As may be expected from the fact that TCDD was discovered to be a teratogen because of its presence as an impurity in 2,4,5-T, TCDD has emerged as one of the most potent animal teratogens known. The mouse appears to be the most sensitive species. Studies by several groups of investigators (78, 79, 91, 92) concur that TCDD is teratogenic in mice at doses as low as 1 to 3 μg/kg/day. The principal teratogenic effects are cleft palate and kidney anomalies. Kidney abnormalities have been observed even in mouse pups nursing on TCDD-treated mothers (92). Rats are apparently more resistant to the

teratogenic effects of TCDD than mice. In three rat teratology studies, kidney abnormalities and intestinal hemorrhage were observed in one study (78) while in the other two studies (68, 93) only intestinal hemorrhage (usually considered as embryotoxicity rather than teratogenicity) was noted in offspring of rats given as much as 8 to 16 μg/kg/day. The kidney is also the teratogenicity target organ of TCDD in the rabbit (94) whereas in the monkey, TCDD induces abnormal development of soft palate (95). Besides TCDD, a number of other chlorinated dibenzodioxins have been tested for teratogenicity (see Table LI). 2-Chloro-, 2,3- and 2,7-dichloro-, 1,2,3,4-tetrachloro- and octachloro-dibenzo-p-dioxins are all nonteratogenic in the rat (34, 68). Only hexachlorodibenzo-p-dioxin (mixed isomers) exhibits teratogenicity inducing cleft palate and skeletal malformation in rats at a dose level of 100 μg/kg/day (34). Thus, like other toxic effects, the teratogenicity of chlorinated dibenzo-p-dioxin is clearly dependent on the position and number of chlorine substitutions on the dibenzo-p-dioxin nucleus. Two isosteric analogs of TCDD, 2,3,7,8-tetrachlorodibenzofuran and 3,3',4,4'-tetrachloroazoxybenzene have recently been reported to be teratogenic in mice (96).

The potential fetal effect of paternal exposure to 2,4-D, 2,4,5-T and TCDD mixture has recently been studied by Lamb et al. (97). Theoretically, fetal effects might occur through the male if the chemical were transmitted to the female via the seminal plasma resulting in direct exposure to the ova. Groups of male C57BL/6N mice were exposed to mixtures of 2,4-D, 2,4,5-T and TCDD shortly before mating. No teratogenic effects were observed in the offspring sired by these males.

5.2.2.3.3 Carcinogenicity and Structure-Activity Relationships.

5.2.2.3.3.1 CARCINOGENICITY OF 2,4-D, 2,4,5-T AND RELATED COMPOUNDS.

The potential carcinogenicity of 2,4-D, 2,4,5-T and six other related herbicides (isopropyl, n-butyl and isooctyl esters of 2,4-D; dichloroprop and its 2,5-dichloro isomer; and silvex) was evaluated in a 1968 screening study sponsored by U.S. National Cancer Institute (98, 99). Two strains of mice, F_1 hybrids of C57BL/6 x C3H/Anf and C57BL/6 x AKR, were used in this study. The compounds were given either by oral administration (gavage followed by diet) at maximum tolerated doses daily for up to 18 months or by a single subcutaneous injection. The results of this study are summarized in Table LII. With one exception, none of these compounds displayed any significant carcinogenic effects by either route. Only 2,4-D isooctyl ester (97% pure; impurities not specified) caused a significant increase in the incidence (5/17) of reticulum cell sarcomas in female mice of the second strain after a single subcutaneous injection of 21.5 mg/kg of the compound. The compound was not carcinogenic in mice of the first strain by subcutaneous route and in mice of either strain by oral route.

The carcinogenicity of 2,4-D (containing no detectable amount of TCDD) was also tested in Osborne-Mendel rats (73). Groups of 50 rats (25 of each sex) were fed for 2 years diets containing 0, 5, 25, 125, 625 or 1,250 ppm 2,4-D. The total number of rats with tumors at the end of the experiment in the control and the five experimental groups was 15, 14, 18, 20, 23 and 22, respectively. The tumors were randomly distributed among various tissues and were of the types normally found in aging Osborne-Mendel rats. Although statistically significant increase in the incidence of malignant tumors was found in the male high-dose group (6/25 vs. 1/25 for control), the authors (73) concluded that "a carcinogenic effect of 2,4-D has not been shown." In a study by Arkhipov and Koslova (cited in ref. 2), random-bred rats were given an amine salt of 2,4-D mixed in the feed at a concentration equivalent to 1/10

Table LII
Carcinogenicity of 2,4-D, 2,4,5-T and Related Compounds in the Mouse and the Rat

Compound[a]	Species & Strain[b]	Route	Principal Carcinogenicity Targets	References
2,4-D acid	Mouse, X or Y	oral or s.c.	None	(98, 99)
	Rat, Osborne-Mendel	oral	Multiple sites (males)[c]	(73)
2,4-D isopropyl ester	Mouse, X or Y	oral or s.c.	None	(98, 99)
2,4-D n-butyl ester	Mouse, X or Y	oral or s.c.	None	(98, 99)
2,4-D isooctyl ester	Mouse, X or Y	oral	None	(98, 99)
	Mouse, Y	s.c.	Hematopoietic system (females)	(98)
Dichloroprop	Mouse, X or Y	oral or s.c.	None	(98, 99)
2-(2,5-Dichloro-phenoxy)-propionic acid	Mouse, X or Y	oral or s.c.	None	(98, 99)
2,4,5-T acid	Mouse, X or Y	oral or s.c.	None	(98, 99)
	Mouse, XVII/G	oral	None	(100)
	Mouse, C3Hf	oral	Multiple sites (females)	(100)
Silvex	Mouse, X or Y	oral or s.c.	None	(98, 99)
2,4,5-Trichlorophen-oxyethanol	Mouse, Swiss	oral	Liver (males)	(101-103)
Clofibrate	Rat, F344	oral	Liver, pancreas, etc.	(104, 105)

[a]See Table XLVI for structural formulas.

[b]Strain X = (C57BL/6 x C3H/Anf)F_1; strain Y = (C57BL/6 x AKR)F_1.

[c]Not considered carcinogenic.

of the LD_{50}. Two treated rats developed tumors (a mammary fibroadenoma and a hemangioma of the mesenterium) after 27 months and one control rat had a mammary fibroadenoma after 27 months. The significance of this study cannot be assessed due to the incomplete reporting of the data.

Muranyi-Kovacs *et al.* (100) retested 2,4,5-T for possible carcinogenicity in two strains (XVII/G and C3Hf) of mice. No carcinogenic effects were observed in XVII/G mice. In C3Hf mice, however, 2,4,5-T (100 mg/l in drinking water for 2 months followed by 80 ppm in diet for life) caused a significant increase in the incidence of various tumors in treated females (13/25 *vs.* 9/44 control). The tumors observed included 4 hepatomas, 3 leukemias, 3 cervical tumors, 2 skin squamous cell carcinomas and 1 osteosarcoma. Since the 2,4,5-T sample used in this study contained very low level (< 0.05 ppm) of chlorinated dibenzodioxins, the authors (100) attributed the carcinogenic effects to 2,4,5-T *per se* and recommended further testing of this compound in other animal species.

Two compounds structurally related to 2,4-D and 2,4,5-T have been found to be carcinogenic in rodents. 2,4,5-Trichlorophenoxyethanol (TCPE), a component in the herbicide Buvinol, has recently been extensively studied by Sugar, Toth and associates (101-103). Weekly oral administration of maximum tolerated doses (67-70 mg/kg) of the compound to Swiss mice for one year led to a significant increase in the incidence of liver tumors (48-58% for experimental *vs.* 26% for control) in male animals. Although the TCPE sample used was contaminated with trace amounts of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), carcinogenicity studies using TCPE sample with different amounts of TCDD contaminants strongly indicate that TCPE itself is hepatocarcinogenic. Clofibrate (4-chlorophenoxyisobutyric acid ethyl ether) is another structurally related compound which induces tumors in the liver, pancreas and in a

number of other tissues in the rat. The details of these studies have been described in Section 5.2.1.7.9 (p. 651 in Volume IIIA). It is important to note that there is some epidemiological evidence that clofibrate (106) and a number of chlorophenoxy acids (see Section 5.2.2.3.5.1) are potential human carcinogens.

5.2.2.3.2.2 CARCINOGENICITY OF HALOGENATED DIPHENYL ETHERS AND DIBENZOFURANS.

There is virtually no information on the carcinogenicity of halogenated diphenyl ethers and dibenzofurans. 4-Bromophenyl phenyl ether was tested in the lung adenoma assay by Theiss et al. (107), using male strain A/St mice as the test species. Multiple injections of the compound up to total doses of 920-3,600 mg/kg body weight had no significant effect on the incidence or multiplicity of lung adenomas. Whereas the study may suggest a lack of carcinogenic activity of the compound toward the mouse lung, no conclusion can be made regarding its carcinogenicity toward other organs. No chlorinated dibenzofurans have thus far been tested for possible carcinogenicity at the time of this writing despite the fact that they are structurally closely related to chlorinated dibenzo-p-dioxins. The unsubstituted parent compound, dibenzofuran, was tested by skin painting in a small number of stock mice; no carcinogenic activity was detected (108).

5.2.2.3.3.3 CARCINOGENICITY OF TCDD AND RELATED COMPOUNDS.

Carcinogenicity Studies on TCDD.

Investigation of the possible carcinogenicity of TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) was prompted by the report of Tung (10), indicating increased liver cancer incidence among Vietnamese exposed to TCDD-contaminated defoliant, "Agent Orange." To date, six carcinogenicity studies (not includ-

ing promotor or modifier studies) on TCDD have been reported; the major findings of these are summarized in Table LIII. Van Miller et al. (116) were among the first to report an increased incidence of tumors in rats exposed to low levels of TCDD. In this study, groups of 10 male Sprague-Dawley rats were fed diets containing 0.001, 0.005, 0.05, 0.5, 1 and 5 ppb TCDD for up to 78 weeks. The number of tumor-bearing rats at the termination of the experiment was 0, 5, 3, 4, 4 and 7, respectively; none of the 10 control rats bore tumors. With the exception of the 5 ppb group, the tumors appeared to be randomly distributed among various tissues suggesting that TCDD may be a promotor of neoplastic changes rather than a complete carcinogen. In the 5 ppb group, six liver tumors (2 cholangiosarcomas and 4 neoplastic nodules) and four pulmonary squamous cell tumors were found among the seven tumor-bearing rats. Thus, the results indicate that TCDD may bring about an increase of tumor incidence in rats at dietary levels as low as 0.005 ppb (equivalent to weekly exposure of 0.001 μg/kg body weight), but the induction of tumors in specific organs (i.e., liver and lung) requires relatively high (5 ppb) dose levels.

The carcinogenicity of TCDD in Sprague-Dawley rats has been confirmed by Kociba et al. (114, 115) in a 2-year ingestion study. However, statistically significant increases in tumor incidences were observed only in rats exposed to 0.1 μg/kg/day. The significant neoplasms include: hepatocellular carcinoma (11/49 experimental vs. 1/86 control) and squamous cell carcinoma of the lung (7/49 experimental vs. 0/86 control) in females; stratified squamous cell carcinoma of the tongue (3/50 experimental vs. 0/85 control) in males; and stratified squamous cell carcinoma of the hard palate/nasal turbinate (8/99 experimental vs. 0/171 control) in rats of either sex. In rats given 0.01 μg/kg/day TCDD, the only significant effect was an increase in the inci-

Table LIII
Carcinogenicity of TCDD and Related Compounds in the Mouse and the Rat

Dibenzo-p-dioxin Derivative	Species & Strain	Route	Principal Organs Affected	References
Unsubstituted compound	Mouse, Swiss Webster	Topical	None? (preliminary)[a]	(109)
	Mouse, $B6C3F_1$	Oral	None	(110)
	Rat, Osborne-Mendel	Oral	None	(110)
2,7-Dichloro-	Mouse, Swiss Webster	Topical	None? (preliminary)[a]	(109)
	Mouse, $B6C3F_1$	Oral	Liver, hematopoietic system (males)[b]	(111)
	Rat, Osborne-Mendel	Oral	None	(111)
2,3,7,8-Tetra-chloro-	Mouse, Swiss Webster	Topical	Skin (females)	(112)
	Mouse, Swiss	Oral	Liver (males)	(103)
	Mouse, $B6C3F_1$	Oral	Liver (males and females); thyroid gland (females)	(113)
	Rat, Sprague-Dawley	Oral	Liver, lung (females); hard palate/nasal turbinates, tongue (males and females)	(114, 115)
	Rat, Sprague-Dawley	Oral	Liver, (high dose); multiple sites (low dose)	(116)
	Rat, Osborne-Mendel	Oral	Thyroid gland (males); liver (females)	(113)
Hexachloro- (1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9- isomers)	Mouse, Swiss Webster	Topical	None	(117)
	Mouse, $B6C3F_1$	Oral	Liver (males and females)	(118)
	Rat, Osborne-Mendel	Oral	Liver (females)	(118)
Octachloro-	Mouse, Swiss Webster	Topical	None (preliminary)[a]	(109)

[a]The data of these studies are considered inconclusive by U.S. National Cancer Institute/National Toxicology Program.

[b]Suggestive evidence of a carcinogenic effect (see text).

dence of hepatocellular hyperplastic nodules (18/50 experimental vs. 8/86 control). At the dose level of 0.001 ug/kg/day, no adverse effects were noted.

In a recent carcinogenesis bioassay of TCDD conducted for U.S. National Toxicology Program (113), Osborne-Mendel rats were given TCDD by gavage at weekly doses of 0.01, 0.05 or 0.5 μg/kg body weight for 104 weeks. Significant neoplastic changes occurred only in the high dose group, showing increased incidences of follicular cell adenomas of the thyroid gland (10/50 experimental vs. 1/69 control) and of hepatocellular neoplastic nodules (12/49 experimental vs. 5/75 control) observed in male and female rats, respectively. Thus, the liver and the thyroid gland appear to be the target organs of TCDD in Osborne-Mendel rats.

The carcinogenicity of TCDD has also been demonstrated in mice. Toth et al. (103) administered TCDD (0.007, 0.7 and 7.0 μg/kg body weight) to Swiss mice by gavage once a week for one year and observed the animals for the rest of their lifespan. A significant increase (21/44 experimental vs. 7/38 control) in the incidence of liver tumors was noted in the medium dose group. Mice in the high dose group had considerably shortened lifespan because of severely ulcerating skin lesions and lethal amyloidosis. In both the gavage and dermal studies of the U.S. National Toxicology Program (112, 113), TCDD was found to be carcinogenic in mice at or close to the maximum tolerated doses. In the gavage study (113), $B6C3F_1$ mice were given weekly doses of 0.01, 0.05 or 0.5 μg/kg (males) and 0.04, 0.2 or 2 μg/kg (females) TCDD for 104 weeks. Significantly higher incidences of hepatocellular carcinomas (males: 17/50 experimental vs. 8/73 control; females: 5/46 experimental vs. 0/69 control) were observed in the high dose groups. In female mice, follicular cell adenomas of the thyroid gland also occurred at signifi-

cantly higher incidence in the high dose group (5/46 experimental vs. 0/69 control). In the dermal study (112), Swiss-Webster mice received topical applications of 0.001 ug (males) or 0.005 ug (females) TCDD, 3 times per week for 99 or 104 weeks. The only significant finding was the induction of skin tumors located on the back at or near the site of application. Most of the skin tumors were fibrosarcomas with an occasional fibroma or keratoacanthoma. The incidence of fibrosarcomas of the integumentary system was significantly higher in female mice (8/27 experimental vs. 2/41 control). An increase in this type of tumor was also noted in male mice (6/28 experimental vs. 3/42 control) but the increase was not statistically significant.

Thus, the totality of the studies indicates that TCDD is carcinogenic in at least two animal species; however, doses close to the maximum tolerated dose (MTD) are often required for the manifestation of its carcinogenic activity. Depending on the species or strain of the animal, the principal target organs are the liver, the lung, the thyroid gland, and nasal/oral cavities by oral administration; the skin appears to be the only affected tissue by dermal route.

Carcinogenicity Studies on TCDD-Related Compounds.

Besides TCDD, the unsubstituted parent compound (dibenzo-p-dioxin) and several other chlorinated derivatives have been tested for possible carcinogenicity by the U.S. National Cancer Institute/National Toxicology Program (see Table LIII). In 1973, King et al. (109) reported the preliminary data of a skin carcinogenesis study on the unsubstituted parent compound and the 2,7-dichloro- and octachloro- derivatives. Swiss-Webster mice were topically treated with 0.2 ml of acetone solutions containing 0.2, 3.0 and 80 mg/ml of the test compound, three times per week. None of these compounds exhibited

any evidence of "complete" carcinogenicity toward the skin by the 59-64th week of the 78 week study. Among the mice that were histopathologically examined, a malignant lymphoma of the lymphocytic type was found in a mouse treated with unsubstituted dibenzo-p-dioxin; two mice treated with octachlorodibenzo-p-dioxin bore subcutaneous tumors. The final results of these studies are not available and are listed as "data inconclusive" in the June 15, 1982 issue of the "Carcinogenesis Testing Program --Chemicals on Standard Protocol" released by the U.S. National Toxicology Program.

Dibenzo-p-dioxin and its 2,7-dichloro derivative have also been tested in feeding studies (110, 111). Osborne-Mendel rats and $B6C3F_1$ mice were given diets containing 5,000 or 10,000 ppm of the respective compound for 110-117 weeks (rats) or 91-101 weeks (mice). No compound-related carcinogenic effect was observed with dibenzo-p-dioxin. The 2,7-dichloro derivative was also concluded to be noncarcinogenic in rats of either sex and in female mice. However, in male mice, the combined incidence of leukemia and lymphoma was significantly higher in the low dose group (7/50 experimental vs. 0/50 control) and the combined incidences of hepatocellular adenomas and carcinomas were also significantly increased (control, 8/49; low dose, 20/50; high dose, 17/40). Thus, the data suggest that 2,7-dichlorodibenzo-p-dioxin may be carcinogenic for male $B6C3F_1$ mice.

A 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-isomers of hexachlorodibenzo-p-dioxin has been tested for possible carcinogenicity in Osborne-Mendel rats and $B6C3F_1$ mice by gavage twice weekly for 104 weeks (118). The doses administered were 1.25, 2.5 or 5 μg/kg/week for rats and male mice and 2.5, 5 or 10 μg/kg/week for female mice. No compound-related tumors were observed in male rats. In female rats, however, the hexachlorodibenzo-p-dioxins induced a significantly increased incidence of hepatocellular neoplastic nodules or

carcinoma (combined incidences: control, 5/75; low dose, 10/50; mid dose, 12/50; high dose, 30/50). In mice of either sex, dose-related increases in the incidences of hepatocellular carcinomas or adenomas (males: control, 15/73; low dose, 15/50; mid dose, 14/49; high dose 24/48; females: control, 3/73; low dose 4/48; mid dose, 6/47; high dose, 10/47) were observed. In direct comparison to the control groups, the increased incidences in the high dose groups were statistically significant. Thus, the hexachlorodibenzo-p-dioxin isomer mixture is carcinogenic in female rats and in mice of either sex, the liver being the only carcinogenicity target organ. In contrast to the gavage study, no carcinogenic effects were noted in a dermal study in which Swiss Webster mice were skin-painted with 0.01 μg of the hexachlorodibenzo-p-dioxin mixture 3 times per week for 104 weeks (117).

The data reviewed above allow a ranking of the carcinogenic potencies of the above compounds and of TCDD. Considering the doses required to elicit significant carcinogenic effects and the incidences or types of tumors, the carcinogenic potency of the dibenzo-p-dioxins appears to follow the order: 2,3,7,8-tetrachloro- > hexachloro- mixture > 2,7-dichloro- > unsubstituted compound. Interestingly, this relative order of carcinogenic potency correlates with the relative order of toxicity or biochemical effects such as enzyme induction suggesting a possible commonality in the mechanisms of action.

5.2.2.3.3.4 MODIFICATION OF CARCINOGENESIS BY TCDD.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been extensively tested for its ability to act as a tumorigenesis promotor, tumor-initiator or cocarcinogen. Somewhat conflicting results have been reported. It appears that the modifying effect of TCDD is dependent on the type of carcinogen admini-

stered and the target organ involved. In some cases, TCDD appears to inhibit rather than to enhance the carcinogenic effects of other chemicals.

In two-stage skin carcinogenesis studies using CD-1 mice, DiGiovanni et al. (119) showed that TCDD (2 μg per mouse) was at most a weak tumor initiator, inducing papillomas in only 14% of the mice with an average of 0.1 papilloma/mouse after promotion for 32 weeks with 12-O-tetradecanoylphorbol-12-acetate (TPA). When applied concurrently with the known tumor-initiator, 7,12-dimethylbenz[a]anthracene (DMBA), TCDD only slightly enhanced the initiating activity of DMBA in an additive manner. Berry et al. (120) found TCDD to be completely inactive as a tumorigenesis promotor in DMBA-initiated mice when applied one week after DMBA at a dose of 0.1 μg per mouse twice weekly for 30 weeks. Interestingly, when applied shortly before DMBA initiation and subsequent promotion by TPA, TCDD (0.1-2 μg per mouse) exhibited a potent anticarcinogenic effect (121-123). The anticarcinogenic effect was both time- and dose-dependent. Maximum inhibition (89-97%) was observed with TCDD administered 3-5 days prior to DMBA initiation. When applied 5 minutes before DMBA, no inhibition was noted. A similar anticarcinogenic effect of TCDD has been found in studies using benzo[a]pyrene or 3-methylcholanthrene as the tumor initiator (123, 124). It has been suggested (121, 124) that TCDD exerts its anticarcinogenic effect by inducing epidermal monooxygenase enzymes that detoxify hydrocarbons or alter the type of hydrocarbon-DNA adduct.

At variance with the above data, the study of Kouri et al. (125) indicates that TCDD (1 or 100 μg/kg), administered intraperitoneally two days prior to a single subcutaneous injection of 3-methylcholanthrene, appears to have no appreciable modifying effect on the induction of subcutaneous tumors by the hydrocarbon in $B6C3F_1$ and D2 mice. When administered simultaneously, however, TCDD significantly potentiated the carcinogenic effect of 3-methyl-

cholanthrene in D2 mice. The authors (125) suggested that TCDD may act as a cocarcinogen possibly by inducing epidermal aryl hydrocarbon hydroxylase. No consistent cocarcinogenic effect was noted in $B6C3F_1$ mice.

In a two-stage hepatocarcinogenesis study, Pitot et al. (126) found that TCDD is a potent promoting agent for diethylnitrosamine-induced hepatocarcinogenesis. In this study, female Charles River rats were partially hepatectomized and exposed to a single initiating dose (10 mg/kg) of diethylnitrosamine followed by biweekly subcutaneous injections of 0.14 or 1.4 μg/kg TCDD for seven months. No hepatocellular carcinoma and few preneoplastic foci occurred in rats receiving diethylnitrosamine or TCDD alone. In the groups that received both the nitrosamine and TCDD, increased incidences of hepatocellular foci, neoplastic nodules (low dose group: 3/5; high dose group: 1/7), and carcinomas (high dose group: 5/7) were observed. The potent promoting activity of TCDD led the investigators (126) to suggest that the hepatocarcinogenic effect observed in chronic studies of TCDD (see Section 5.2.2.3.3.3) may arise from its promoting activity rather than its activity as a complete carcinogen.

5.2.2.3.4 Metabolism and Mechanism of Action.

Halogenated phenoxy acid derivatives are absorbed readily from the gastrointestinal tract of humans or animals after oral dosing (e.g., 127, 128), but slowly upon dermal contact (129). The pharmacokinetics of the salt or ester forms of phenoxy acids is similar to those of the free acids (e.g., 4, 128-130) because of rapid hydrolysis (131). Owing to their relatively high hydrophilicity, these compounds are rapidly excreted, mostly unchanged, in the urine (127-130). There is some evidence that 2,4-D and 2,4,5-T are rapidly excreted through an active organic anion transport system in the renal proximal tubules (132, 133); this renal transport system may, however, be

saturated or impaired upon exposure to high doses (4, 134). Biotransformation of halogenated phenoxy acids does not seem to occur to any significant extent in mammalian species. The usual metabolic routes of most aralkoxy compounds, ring hydroxylation and cleavage at the ether linkage, do not appear to occur to any significant extent for phenoxy acids (reviewed in ref. 4). There is some suggestive but unconfirmed evidence of the possible formation of 4-chloro-o-cresol from MCPA in the rat (135). Depending on the dose applied, a portion of halogenated phenoxy acids may be excreted as conjugates, mainly with glycine, taurine or glucuronic acid (reviewed in ref. 4).

The mode of herbicidal action of halogenated phenoxy acids suggests that these compounds may also be potentially genotoxic to animal cells if sufficient amount reaches the genome. In vitro studies using cultured chicken muscle cells revealed that non-cytotoxic concentrations of 2,4-D inhibit cell differentiation and cell mitosis, probably by affecting the template activity of the deoxyribonucleoprotein matrix (136). Bednar et al. (137) showed that 2,4-D may be activated by a H_2O_2-peroxidase system to electrophilic reactive intermediate(s) (possibly an epoxide) that bind covalently to soluble RNA in an in vitro system. Differential labeling studies suggest that only the benzene ring moiety is incorporated whereas the acetic acid moiety is not. This activation is considered to be the mechanism whereby the ultimate phytohormonal effects in plants are produced. Conceivably, the same mechanism can be potentially genotoxic in mammalian tissues. It remains to be investigated whether mammalian tissues are capable of activating chlorophenoxy acids in this manner. A number of chlorophenols, the potential metabolites of chlorophenoxy acids, have been shown to be carcinogenic in animal bioassays. The details of these studies are discussed in Section 5.2.2.5. Vainio et al. (138) have recently demonstrated that, like clofibrate (see Section

5.2.1.7.9), 2,4-D and MCPA are capable of inducing peroxisome proliferation in Chinese hamsters. Clofibrate has been suggested to exert its carcinogenic action through excessive production of intracellular hydrogen peroxide causing DNA damage. It is probable that a similar mechanism may account for the carcinogenic action of other chlorophenoxy acids.

Relatively little is known about the metabolism of TCDD (reveiwed in ref. 139). Earlier pharmacokinetic studies in rats (140-143), mice (144), guinea pigs (145) and monkeys (142) indicate that TCDD is excreted very slowly, suggesting relatively high metabolic stability of the compound. Olson et al. (41) showed that the Syrian golden hamster, which is unusually resistant to TCDD toxicity, excretes TCDD in the urine and feces at a much faster rate than other species. High-pressure liquid chromatographic analysis of urine and feces indicated the presence of water-soluble metabolites. No metabolites were found in extracts of liver or adipose tissue, suggesting that the metabolites are readily excreted in the urine and bile and that metabolism may be the rate-limiting step for excretion. A recent study by Ramsey et al. (146) indicates that TCDD is also metabolized in rats to more polar metabolites which are excreted as such or as conjugates of glucuronide. Using an in vitro system with isolated rat hepatocytes, Sawahata et al. (147) isolated two polar metabolites and identified them as 1-hydroxy-2,3,7,8-tetrachlorodibenzo-p-dioxin and 8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin.

The mechanism of carcinogenic action of TCDD and related compounds is poorly understood. An in vivo covalent binding study by Poland and Glover (148) indicated that ^{3}H-labeled TCDD does not bind to rat liver DNA or ribosomal RNA to any significant extent. The maximum estimate (assuming that all unextractable radioactivity represents covalent binding) of covalent binding of TCDD to DNA is less than 1 molecule of TCDD per 10^{11} nucleotides, or 4 to 6

orders of magnitude lower than that observed for most chemical carcinogens. As discussed in Section 5.2.2.3.2.2, despite the favorable molecular size and shape of TCDD as an intercalator, there is no convincing evidence that TCDD is mutagenic. At least three alternative mechanisms have been hypothesized (148): (a) TCDD is a potent inducer of microsomal mixed function oxidases, and this induction might enhance the rate of formation of reactive intermediates from potentially carcinogenic endogenous, dietary and environmental compounds, (b) TCDD may act as a promotor and stimulate previously initiated cells to divide, and (c) TCDD may act in a hormone-like manner, trophically stimulating the target tissues. As discussed in Section 5.2.2.3.3.4, the finding of Pitot et al. (126) that TCDD is a potent promotor of diethylnitrosamine-induced hepatocarcinogenesis supports hypothesis (b). However, TCDD is devoid of tumorigenesis promoting activity in skin carcinogenesis studies using 7,12-dimethylbenz[a]anthracene as the tumor-initiator (120); in fact, under some circumstances, TCDD appears to inhibit rather than to enhance the carcinogenic effects of a number of skin carcinogens (see above in this Section). In addition to the hypothesized mechanisms discussed above, it should be pointed out that TCDD is a potent immuno-suppressant in several species of animals; an impairment of immune competence is often associated with an enhancement of tumor development.

5.2.2.3.5 Environmental Significance.

5.2.2.3.5.1 EPIDEMIOLOGIC EVIDENCE.

The potential carcinogenic risk of human exposure to halogenated phenoxy acids, dibenzofurans and dibenzodioxins has been a subject of great interest and concern in recent years. Studies by a number of Swedish investigators indicate that human exposure to phenoxy acids may be associated wih an

increase in the risk for several types of tumors, although firm epidemiologic evidence of a causal relationship to specific compounds is still lacking.

In 1977, Hardell (149) noted that several Swedish patients with soft-tissue sarcomas were reported to have had previous heavy exposure to chlorinated phenoxy acids. Phenoxy acid herbicides (mainly 2,4-D, 2,4,5-T and MCPA) have been used to control unwanted hardwoods or weeds in Swedish forestry and farming since the beginning of the 1950's. A subsequent matched case-control study by Hardell and Sandstrom (150) of 52 patients (from northern Sweden) with a known history of exposure to chlorinated phenoxy acids or phenols revealed an approximately 6-fold increase in the risk for this type of tumor. It was not known, however, whether impurities (such as chlorinated dibenzodioxins and dibenzofurans) present in these herbicide preparations played any contributory role. A more recent case-control study, by Eriksson et al. (151), on patients in southern Sweden indicated about the same extent of increase in risk for soft-tissue sarcomas. Moreover, persons exposed to 2,4-D, MCPA, dichloroprop and mecoprop (these phenoxy acids are generally not considered to be contaminated by chlorinated dibenzodioxins or dibenzofurans) appeared to have approximately the same degree of increased carcinogenic risk as those exposed to 2,4,5-T, suggesting that the phenoxy acids per se may be the suspected carcinogen.

Besides soft-tissue sarcomas, human exposure to phenoxy acids or chlorophenols may also be associated with an increased risk to non-Hodgkin's malignant lymphoma of the histiocytic type. Hardell (152) first reported that among 17 Swedish patients admitted for treatment of histiocytic lymphoma, 11 reported such exposure. The median latent period was 15 years. A matched case-control study by Hardell et al. (7) gave a calculated relative risk of 4.8 for phenoxyacetic acids. Olsson and Brandt (153) reviewed the case

histories of 123 patients with non-Hodgkin's lymphoma and found 5 patients with cutaneous lesions as the sole clinically detectable manifestation of the malignancy. Four of these 5 patients have one commonality -- repeated skin exposure to 2,4-D, 2,4,5-T or MCPA for a period of 18-20 years. Axelson et al. (8) followed a cohort of 348 Swedish railroad workers who were exposed to herbicides, phenoxyacetic acids and/or amitrol, during the period 1957-1972. An excess of stomach cancer (3 observed cases versus 0.41 expected) was noted among those exposed to phenoxyacetic acids. An increased mortality from lung cancer was also reported among a number of German pesticide workers who were exposed to 2,4-D and MCPA, as well as other pesticides (154). Another chlorinated phenoxy acid derivative, which has been implicated as being potentially carcinogenic in humans (106, 155) is clofibrate, a drug that has been used for the treatment of hyperlipoproteinemia in the United States and Europe (see Section 5.2.1.7.9 and "Notes added after completion of Section 5.2.1.7" in Vol. IIIA). Increased incidence of cancer of the liver, gall bladder and intestines were reported in patients treated with clofibrate (106). The totality of these data suggest that further epidemiologic studies are needed and that chlorinated phenoxy acid derivatives must be handled with caution irrespective of whether they are contaminated with chlorinated dibenzodioxins or not.

Epidemiologic studies on potential carcinogenic risk of human exposure to chlorinated dibenzodioxins are mostly incomplete at the time of this writing. Tung (10) reported an increase in the incidence of primary carcinoma of the liver among cancer patients admitted to hospitals in Vietnam during the period 1962-1968. He attributed this increase to TCDD exposure as a result of the spraying, for military purposes, of defoliant (later found to be contaminated with TCDD) in Vietnam during the 1960's. No epidemiologic data were,

however, provided in this study. Jirásek et al. (cited in ref. 2) followed up 55 subjects of a cohort of 78 TCDD-exposed Czechoslovakian workers for 5-6 years and observed two cancer deaths, both from bronchogenic carcinoma. Based on 1965 World Health Organization lung cancer mortality rate for Czechoslovakia, the expected number of lung cancer deaths for a cohort of this size would be 0.12 (2). Thiess and Goldmann (cited in ref. 2) noted 4 cancer deaths (1 lung, 2 gastric and 1 colonic carcinoma) among 53 TCDD-exposed German workers; the study is still in progress. Zack and Suskind (156) followed, over a period of nearly 30 years, 121 workers who developed chloracne resulting from TCDD exposure in a 2,4,5-trichlorophenol process accident at a chemical plant in Nitro (West Virginia, USA) in 1949. Analysis of the mortality data indicated no apparent excess in deaths from malignant neoplasms (9 observed versus 9.04 expected). There were 5 lung cancer deaths versus 3.02 expected and 1 skin cancer death versus 0.15 expected. The investigators cautioned that the results of this study cannot be considered conclusive because of the small size of the cohort and the relative small number of deaths observed. Bishop and Jones (157) reported two cases (compared to 0.28 cases expected) of non-Hodgkin's lymphoma of the skin among 158 workers in a plant which manufactured pentachlorophenol and its sodium salt. Chlorinated dibenzo-p-dioxins, particularly the octachloro and the hexachloro congeners, occurred as contaminants up to about 200 ppm at the intermediate stages of manufacture and at 5 ppm in the final products. The tragic accident of massive release of TCDD and related compounds into the atmosphere of Seveso (Italy) in 1976 attracted a flurry of epidemiologic studies (e.g., 72, 158; see also ref. 159). Thus far, data have not revealed any evidence for carcinogenicity (72). However, in view of the fact that the long latent periods are usually needed for tumor development, it is premature to draw any conclu-

sion at this stage. The U.S. National Institute for Occupational Safety and Health (NIOSH) is currently conducting an extensive epidemiologic study involving about 3,000 chemical workers who had been or were suspected to have been exposed to TCDD; the results of this study are not expected to be released until the mid-1980's.

5.2.2.3.5.2 ENVIRONMENTAL SOURCES, OCCURRENCE AND EXPOSURE.

Human exposure to chlorophenoxy acids is mainly associated with the use of these compounds as herbicides. A number of chlorophenoxy acids, particularly 2,4-D and MCPA, are still widely used to control broad-leaf weeds (2-4). Apart from occupational exposure, the population-at-large may be seasonally exposed during lawn spraying. Chlorophenoxy acids are not known to persist in the environment; human exposure from the general environment appears to be limited. The possible occurrence of chlorophenoxy acids in the finished drinking water of U.S. cities has been monitored by the U.S. Environmental Protection Agency (160). In a 10-city survey, trace amounts of 2,4-D (40 nanogram/liter) and silvex (20 nanogram/liter) were detected in the drinking water of one city. An expanded survey of 53 cities in the mid-west region indicated the presence of 2,4-D in the raw water of only one city; none was detected in the finished drinking water. The U.S. Food and Drug Administration (161-163) has monitored the level of 2,4-D, 2,4-DB and 2,4,5-T in the average American diet in its "Market Basket" study. With one exception, no significant amounts of any of these herbicides were detected in 12 different categories of foodstuffs destined to adults, infants and "toddlers" (young children). In one case, trace amounts of 2,4-D (0.025 ppm) were detected in the "sugar and adjuvant" category of toddler diet. Based on this information, the possible daily intake of 2,4-D by toddlers was estimated to be 0.0058 μg/kg body weight (163). Another minor source of exposure is from

the use of clofibrate for the treatment of hyperlipoproteinemia (see Section 5.2.1.7.9).

The intensity of concern over the environmental exposure to halogenated dibenzo-p-dioxins and dibenzofurans is generated by the extreme toxicity of some members of these two series of compounds, their frequent occurrence in certain industrial chemicals and associated wastes and their persistence in the environment. Detailed reviews on environmental sources and occurrence and on exposure to halogenated dibenzo-p-dioxins and dibenzofurans (17, 19, 22, 23, 164, 165) have been published. A brief perspective on these topics is given below.

Chlorinated dibenzo-p-dioxins have long been known to occur as unwanted byproducts in chemical manufacturing processes involving chlorinated phenols. Figure 21 depicts the pathways for the synthesis of 2,4,5-trichlorophenol, the herbicide 2,4,5-T and the bactericide hexachlorophene. The conversion of 1,2,4,5-tetrachlorobenzene to 2,4,5-trichlorophenate is known to be an accident-prone reaction; at temperatures above 230°C, dangerous exothermic decomposition reaction may occur leading to further increase in temperature and gas formation. At temperatures above 180°C, 2 molecules of 2,4,5-trichlorophenate may combine to form the highly stable TCDD. Similarly, a variety of other chlorinated dibenzo-p-dioxins may be pyrolytically formed from other chlorophenols, with at least one chlorine and one phenolic group substituted ortho to each other (e.g., 2,7-dichlorodibenzo-p-dioxin from 2,4-dichlorophenate; octachlorodibenzo-p-dioxin from pentachlorophenate) (see 22, 164, 165). Chlorinated diphenyl ethers are also precursors of chlorinated dibenzo-p-dioxins (165, 166); chlorinated diphenyl ethers with a chlorine atom and a phenolic group at positions ortho to the intercyclic bond are often called "pre-dioxins" and are believed to be an intermediate in the thermal

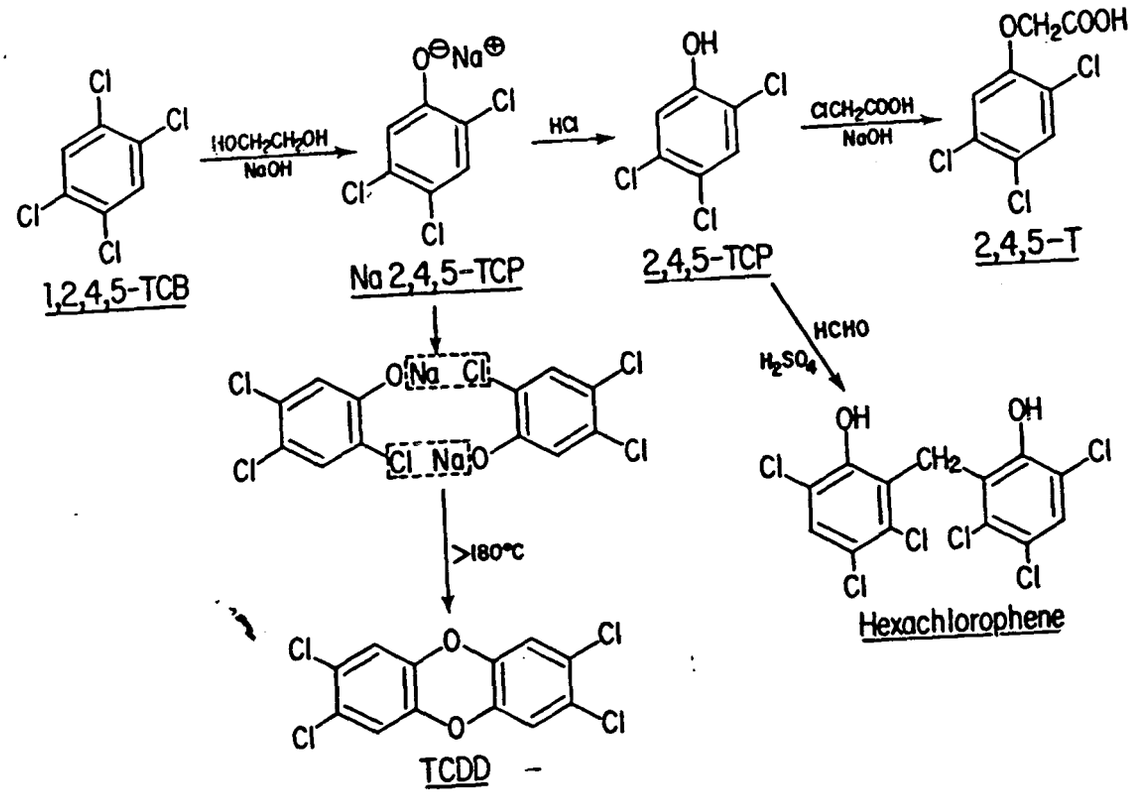


Fig. 21. Formation of TCDD during synthesis of 2,4,5-TCP, 2,4,5-T or hexachlorophene. The abbreviations used are: 1,2,4,5-TCB = 1,2,4,5-tetrachlorobenzene; Na 2,4,5-TCP = 2,4,5-trichlorophenol sodium salt (sodium 2,4,5-trichlorophenate); 2,4,5-TCP = 2,4,5-trichlorophenol; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; TCDD = 2,3,7,8-tetrachloro-dibenzo-p-dioxin.

synthesis of chlorinated dibenzo-p-dioxins from chlorophenols. A number of commercially used chemical processes with a reasonable potential for dioxin byproduct contamination have been surveyed and assessed (167).

The mechanism of formation of halogenated dibenzofurans has been reviewed in details by Choudhry and Hutzinger (165). Morita et al. (168) showed that heating a polychlorinated biphenyls (PCBs) mixture for one week at temperatures between 270°C and 300°C gives rise to a variety of chlorinated dibenzofurans, including up to 80 ppm of the highly toxic 2,3,7,8-tetrachlorodibenzofuran. Pyrolysis of individual PCB congeners leads to the formation of specific chlorinated dibenzofurans via intramolecular cyclization; for example, 2,4,5,3',4'-pentachlorobiphenyl has been shown to be a precursor of 2,3,6,7- and 2,3,7,8-tetrachlorodibenzofuran (169). Chlorinated diphenyl ethers are also excellent precursors for chlorinated dibenzofurans; yields as high as 4.5% were obtained in the pyrolysis of these precursors at 600°C (166). Dibenzofurans may be formed during thermal cracking of phenols or cresols (170); Buser (171) postulated that pyrolysis of chlorobenzenes may generate chlorinated dibenzofurans via chlorophenols.

There are numerous reports of occurrence on the chlorinated dibenzo-p-dioxins and dibenzofurans in various industrial and commercial products. An average of 1.86 ppm TCDD (maximum 47 ppm) was found in surplus Agent Orange (1:1 mixture of 2,4,5-T and 2,4-D) preparations stockpiled after the Vietnam War (14). A survey by Woolson et al. (172) of 42 samples of 2,4,5-T manufactured from 1966 to 1970 showed that 13 of these samples contained 10-100 ppm TCDD, 7 had less than 10 ppm and 22 had less than 0.5 ppm. Commercial chlorophenols also contain various chlorinated dibenzo-p-dioxin congeners (22). Some pentachlorophenol products manufactured before 1970 contained as much as 3,600 ppm octachlorodibenzo-p-dioxin (see ref. 22). An exhaustive list of

commercial products that may be contaminated with chlorinated dibenzo-p-dioxins has been compiled by Esposito et al. (22). Since the time that the highly hazardous nature of TCDD was widely publicized, manufacturers have significantly reduced the TCDD content in various products. The 2,4,5-trichlorophenol produced around 1977 in the United States reportedly contained an average of 0.026 ppm TCDD (173). Chlorinated dibenzofurans have been detected in various commercial samples of polychlorinated biphenyls (PCBs) as well as in the tissues of individuals ("Yusho" disease) who had been exposed to PCBs (174-176; see also refs. 17, 169).

Human exposure to chlorinated dibenzofurans and dibenzo-p-dioxins may occur in the workplace as well as in nonoccupational settings. Since 1949, over 200 TCDD-related industrial accidents have occurred around the world (9). A partial list of reported incidents of occupational exposure to chlorinated dibenzo-p-dioxin during routine chemical manufacturing has been compiled by Young et al. (177). Industries or occupations using chemicals which may be contaminated with chlorinated dibenzo-p-dioxins or dibenzofurans include textiles, leather tanning, wood preserving, pulp and paper, pesticide formulators and applicators, automotive, construction, drug and cosmetics, paint, farming, railroad maintenance, transformers and capacitors manufacturing or repairing, chemical laboratories and waste management (17, 22). In addition, a group of U.S. military personnel may have been exposed to TCDD during handling and spraying of Agent Orange in the Vietnam War. As mentioned in Section 5.2.2.3.5.1, the U.S. National Institute for Occupational Safety and Health is currently conducting an extensive epidemiologic study of potential long-term health hazards of occupational exposure to TCDD.

The general public may be exposed to chlorinated dibenzo-p-dioxins or dibenzofurans in localized areas in the vicinity of industrial or transporta-

tion accidents, in areas where contaminated industrial wastes were improperly disposed or in areas where contaminated herbicides were sprayed. There are a number of well-publicized episodes of such exposures (reviewed in ref. 22). In 1976, a massive amount (estimted to be somewhere between 300 g and 130 kg) of TCDD was released into the atmosphere over an area of about 700 acres in Seveso, Italy during an industrial accident. Numerous investigations have been devoted to studying the toxicological effects caused by TCDD contamination (23, 72, 158, 159, 178-180). Thus far, the most evident acute pathology is the induction of chloracne, especially in children. Although there are some reports (72, 158) of the apparent lack of genotoxic effects of TCDD in the exposed population, it is probably still too early to draw any conclusion about the chronic effects of this incident at this time. In 1979, a railroad accident at Sturgeon, Missouri, caused the spillage of a tank car of o-chlorophenol; subsequent analysis of the contents showed the presence of 37 ppb TCDD. The presence of TCDD in o-chlorophenol is somewhat unexpected. Details of the incident have not been released because of pending legal action (22). Leakage of PCBs into rice oil during processing caused an epidemic of toxic effects ("Yusho" disease) in Japan in 1968 (181; see also Section 5.2.2.2); chlorinated dibenzofurans were detected in the contaminated rice oil (181, 182) and in tissues of "Yusho" patients (176). The most notable incident of public exposure to TCDD-contaminated industrial wastes involved the spraying of TCDD-contaminated waste oil to control dust in horse arenas and private roads (183). Up to 100 sites in the state of Missouri may have been contaminated with TCDD since 1971 (184, 185). Chlorinated dibenzo-p-dioxins (including TCDD) have also been found in two chemical landfills in Niagara Falls, New York. One of these, Love Canal, was until recently the site of a residential community. About 30 tons of 2,4,5-trichlorophenol wastes were

reportedly buried in the Love Canal (22). The incineration of TCDD-contaminated chemical wastes has been suspected to contribute to the presence of TCDD in the atmosphere in the vicinity of a chemical plant in Michigan (22). The use of TCDD-contaminated defoliant in Vietnam is another well-publicized incident of public exposure and is the subject of a U.S. Air Force technical report by Young et al. (177). The spraying of 2,4,5-T and silvex in forest areas in Oregon led to a heated public debate and culminated in an emergency ban in 1979 on the continued use of these herbicides in these areas (22). Many other incidents of possible public exposure to TCDD through the use of herbicides have been reported throughout the world (22).

Besides exposure in localized areas, public exposure may also occur from the general environment; relatively litte information is available to assess the extent of such exposure. Miller (186) suggested that a worldwide background of atmospheric TCDD contamination may exist as a result of the incineration of TCDD-contaminated Agent Orange by the U.S. Air Force. Pyrolysis of chlorinated benzenes, phenols, phenoxy acids, biphenyls and aromatic ethers may also generate chlorinated dibenzofurans and/or dibenzo-p-dioxins (166, 169, 187-189; reviewed in refs. 17, 164). There is some suggestion that chlorinated dibenzo-p-dioxins may be formed de novo in the burning of fossil fuel (190); however, the actuality of such a route of formation remains questionable (191-193). Chlorinated dibenzo-p-dioxins have been detected in the water effluents from some chemical plants; however, there appears to be no indication of their possible occurrence in drinking water supplies (22).

There are several reports on the possible contamination of human food sources with trace levels of TCDD. Beef fat taken from cattle grazed on pasture treated with 2,4,5-T occasionally contained TCDD at 3-70 parts per

trillion levels (22, 194, 195). Food samples collected from south Vietnam in 1970 (196) and from Seveso, Italy in 1976 (197) contained higher levels of TCDD. As discussed in Section 5.2.2.2.5.2, PCBs may be present in a variety of foodstuffs. In view of the contamination of PCBs with chlorinated dibenzofurans, it is possible that trace amounts of chlorinated dibenzofurans may also be found in human food sources.

REFERENCES TO SECTION 5.2.2.3

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Noted Added After Completion of Section 5.2.2.3

Several recent monographs on the toxicological and environmental aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds have been published (1-4). The U.S. National Institute for Occupational Safety and Health issued a "Current Intelligence Bulletin" on TCDD (5) and a special issue of Chemical and Engineering News was devoted to discussion on TCDD (6). Reuber (7) has recently written a comprehensive review on the carcinogenicity and toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D).

In contrast to the lack of conclusive evidence for mutagenicity of TCDD in the Ames test, Rogers et al. (8, 9) reported that TCDD is mutagenic in L5178 mouse lymphoma cells. The mutagenic potency and the dose-response curve of TCDD appears to be comparable to those of classical intercalating agents such as proflavin. Tenchini et al. (10) completed a comparative cytogenetic study on maternal and fetal tissues derived from TCDD-exposed and nonexposed pregnant women (during the Seveso incident in Italy), who underwent induced abortions. In agreement with previous reports, no significant clastogenic effects of TCDD on maternal peripheral blood and placental tissues were observed. However, a significant increase was noted in the frequencies of aberrant cells and in the average number of aberrations per damaged cell in the aborted fetal tissues. Although a causal relationship cannot be established, the results suggest a possible transplacental clastogenic action of TCDD. Two nitrated derivatives of dibenzo-p-dioxin (2-nitro- and 2,3-dichloro-7-nitro-) were found to be potent frameshift mutagens in the Ames test (11). Although this type of mutagenic activity was attributed to the aromatic nitro group, the presence of the chlorine substituents made the compound also slightly active as a base-pair substitution type mutagen. An isosteric structural analog of TCDD, 3,4,3',4'-tetrachloroazobenzene, is a weak frameshift

mutagen in the Ames test (12). The less chlorinated homolog, 4,4'-dichloroazobenzene, and its azoxy derivative, 4,4'-dichloroazoxybenzene, are considerably more active, inducing both frameshift and base-pair substitution type mutations.

Additional teratogenicity testing of 2,4-D has been carried out by Rodwell et al. (13) using F344 rats. The animals were given 8, 25 or 75 mg/kg/day 2,4-D orally from day 6 through day 15 of gestation. There was no evidence of teratogenicity. 2,4-Dichlorophenol, a metabolite of 2,4-D was also nonteratogenic (14). In both studies, the highest dose levels were sufficiently high to cause a slight degree of maternal toxicity. Further evidence for the potent teratogenicity and of TCDD and its structural analogs has been obtained in several recent studies. Giavini et al. (15) found that a 2-week exposure of female rats to TCDD shortly before mating (not during gestation) interfers with normal embryofetal development. At a daily dose of 2 ug/kg, a variety of maternal and fetal toxic effects as well as malformations occur, indicating that TCDD accumulated before mating may exert teratogenic/embryotoxic effects during the organogenesis period. D'Argy et al. (16) showed that the teratogenic effect of TCDD in mice is strain-specific. A single dose of 30 ug/kg TCDD on day 12 of gestation induced cleft palate in 75-100% of NMRI mice but had no significant effects in DBA mice (which lack the cytosolic receptor for TCDD). Two isosteric structural analogs of TCDD -- 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 3,3'4,4'-tetrachloroazoxybenzene (TCAOB) -- have been shown to be teratogenic in strain C57B1/6N (17) and NMRI (16) mice, respectively. The spectrum of teratogenicity targets of these two compounds is similar to that of TCDD suggesting a common mechanism of action. The teratogenic potency of TCDF and TCAOB is about 10-30 and 260 times lower than that of TCDD, respectively.

No new animal carcinogenicity studies on 2,4-D, 2,4,5-T and TCDD have been found in the literature since the completion of Section 5.2.2.3. Decabromodiphenyl ether (crude grade; 77.4% pure, contains 21.8% nonabromo- and 0.8% octabromo- homologs), a fully halogenated structural analog of TCDD was reported to be noncarcinogenic in the Sprague-Dawley rats after feeding for 2 years daily doses of 0.01, 0.1 or 1 mg/kg of the compound (18, 19). The only adverse effect noted was an accumulation of bromine in the liver and adipose tissues of rats of the high dose group. The amine salt of 2,4-D was reported to promote 3-methylcholanthrene-initiated skin carcinogenesis in CBA x C57/BL hybrid mice (Archipov and Kozlova, cited in ref. 7). The promoting activity of 2,4-D is relatively weak. Skin papillomas occurred in 17.7% of mice treated with initiating doses of 3-methylcholanthrene (0.5% solution in benzene) followed by 2,4-D (10% solution in acetone); none were found in mice treated with either agent alone. Poland et al. (20) studied the capacity of TCDD to promote skin carcinogenesis in HRS/J mice and found evidence of a genetic control of the susceptibility to TCDD-induced tumor promotion linked to the control of hair growth. Following initiation with 7,12-dimethylbenz[a]anthracene (DBMA), repeated topical application of TCDD failed to produce any skin tumor in HRS/J haired mice. In contrast, the same regimen of DMBA and TCDD treatment produced skin tumors in 15 of 19 HRS/J hairless mice. A similar, dose-dependent promoting effect of TCDD in HRS/J hairless mice was observed using N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) as the initiator. Compared to the classical promotor, 12-O-tetradecanoylphorbol-13-acetate (TPA), TCDD was about 100 times less active. The capacity of several congeners or analogs of TCDD to promote skin tumorigenesis was also tested; the isosteric analogs, TCDF and 3,4,5,3',4',5'-hexabromobiphenyl, were both active promotors whereas 2,7-dichlorodibenzo-p-dioxin and 2,4,5,2',4',5'-hexa-

bromobiphenyl had no activity. The results indicate that TCDD and related halogenated aromatic hydrocarbons comprise a class of potent tumor promotors whose expression is genetically controlled. It is interesting to note that the HRS/J hairless mouse is considered to be a good model for dermatoxicological study of human skin.

A variety of new epidemiological studies have been conducted to assess further the carcinogenic risk of human exposure to 2,4-D, 2,4,5-T, TCDD and related compounds. In contrast to an elevated risk to soft-tissue sarcomas and malignant lymphomas (see Section 5.2.2.3.5.1), Hardell et al. (21, 22) did not find any significant increase in the incidence of colon, nasal and nasopharyngeal cancers in Swedish workers exposed to chlorophenols and chlorophenoxy acids. Combining the mortality data from three separate studies (each reporting a lack of carcinogenic risk) of four small cohorts of U.S. workers involved in the production of 2,4,5-trichlorophenol and 2,4,5-T, Honchar and Halperin (23) noted an unusually high incidence of soft-tissue sarcoma (three cases out of 105 deaths; approximately 43 times higher than expected). Subsequently, four additional cases of soft-tissue sarcomas were reported among U.S. workers who were possibly exposed to chlorophenols, 2,4,5-T or TCDD (see ref. 24). However, a more recent detailed review of work exposure record and re-examination of pathological specimens led to the conclusion that only two of the seven cases had both confirmed exposure to 2,4,5-trichlorophenol or 2,4,5-T and diagnosis of soft-tissue sarcoma (J.D. Millar, cited in ref. 5). Moses et al. (25) reported the health status of 226 U.S. workers in a chemical plant (in Nitro, West Virginia) where 2,4,5-T (believed to be heavily contaminated with TCDD) had been manufactured from 1948 to 1969 and where an explosion of reactor vessel containing 2,4,5-T occurred in 1949. Over 52% of the workers developed chloracne which persisted for an average of 26 years. Based

on worker recall of job assignment, the development of chloracne was positively correlated to the extent of exposure. Twenty-five subjects reported a positive cancer history. Among these, eleven had bladder cancer; however, all had had also an occupational history of exposure to 4-aminobiphenyl, a known bladder carcinogen. Skin cancer was reported by 12 subjects; however, there appeared to be no association between chloracne and skin cancer. Other types of cancers reported included two cases of laryngeal cancer, one case each of kidney, lymphoma, bowel, leukemia, and prostate cancer. No attempt was made in this study to assess whether the occurrence of cancer was related to exposure. An extensive epidemiological investigation ("Project Ranch Hand II") of health effects in U.S. Air Force personnel following exposure to herbicides (most notably Agent Orange) during the Vietnam War has recently been completed (26). A group of 1,241 men who handled herbicides daily for up to 4 years (probably the most heavily exposed group in the Air Force) was selected and matched by age, race and occupational category to a comparison group of non-exposed personnel. The men were subjected to a complete physical examination including an evaluation of several major organ systems and their functions, neurological and psychological assessment. Members of the Ranch Hand (exposed) group did not develop chloracne or porphyria cutanea tarda, two of the most characteristic toxic effects of TCDD. Analyses of cancer data showed significantly more nonmelanotic skin cancer in the Ranch Hand group; however, these analyses have not been adjusted for the possible impact of sunlight exposure, the prime etiology of skin cancers. There were no statistically significant differences in the occurrence of malignant or benign systemic tumors between the two groups. The report concluded that there is insufficient evidence to support a cause and effect relationship between herbicide exposure and adverse health effects in the Ranch Hand group.

The metabolism and disposition of TCDD and related compounds has been further studied. Poiger et al. (27) identified the chemical nature of five polar metabolites of TCDD found in the bile of dogs as 2-hydroxy-3,7,8-trichlorodibenzodioxin, 2-hydroxy-1,3,7,8-tetrachlorodibenzodioxin, and dihydroxytrichlorodibenzodioxin (probably arising from ring epoxidation), and 4,5-dichlorocatechol and dihydroxytetrachlorodiphenyl ether (which may be formed by cleavage of the ether bridge(s)). Once formed, the metabolites appear to be readily excreted with no evidence of bioaccumulation (28) suggesting that metabolism of TCDD may be a rate-limiting step in its elimination. Significant differences in the disposition of TCDD in C57Bl/6J and (C57Bl/6J x DBA/2J)F_1 mice as compared to DBA/2J mice were noted by Gasiewicz et al. (29); the half-life of TCDD in these three strains was estimated to be 11.0, 12.6 and 24.4 days, respectively. The difference may be due, in part, to the sequestration of TCDD in the adipose tissue which is more abundant in the DBA/2J strain. Similar strain differences were observed in the disposition of 2,3,7,8-tetrachlorodibenzofuran (TCDF) although TCDF was excreted at a much faster rate than TCDD (30). The half-life of TCDF was estimated to be around 2 days in C57Bl/6J mice and 4 days in DBA/2J mice.

The ability of chlorophenoxyacetic acids to induce peroxisomal enzymes in the rat liver has been studied by Kawashima et al. (31) prompted by the findings of positive correlation between peroxisome proliferation and carcinogenesis (see Section 5.2.1.7.9 and Notes Added After Completion of Section 5.2.1.7; see also ref. 32). Both 2,4,5-T and, to a lesser extent, 2,4-D were shown to induce peroxisomal β-oxidation. 2,4-D differed from 2,4,5-T in not causing hepatomegaly or catalase induction. The less chlorinated 2-chloro- and 4-chlorophenoxyacetic acid and the unsubstituted phenoxyacetic acid were inactive. Thunberg (33) observed that TCDD treatment leads to substantial

reduction in the level of vitamin A in the liver and serum of a variety of animal species and proposed that the TCDD-induced vitamin A deficiency may play a role in the carcinogenesis or tumor promotion by the compound. Vitamin A has been shown to suppress or prevent chemical carcinogenesis in a number of animal studies; conversely, vitamin A deficiency has been associated with increased susceptibility to carcinogenesis. The mechanism of promotion of carcinogenesis by TCDD has been studied by Boreiko and Dorman (34). Unlike 12-O-tetradecanoylphorbol-13-acetate (TPA), TCDD fails to inhibit intercellular communication in cultured C3H10T1/2 cells. The interference of intercellular communication has been proposed as a mechanism of tumor promotion by a variety of promotors (35).

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