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**Synthesis of Dioxins as Analytical
and
Toxicological Standards**

T. Lee Collier

A thesis submitted to the Faculty of
Graduate Studies in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Department of Chemistry
Carleton University

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of Graduate Studies the acceptance of this thesis
submitted by T. Lee Collier, B. Sc., in partial
fulfillment of the requirements for the degree of
Doctor of Philosophy.

Supervisor

Chairman of the Chemistry Department

External Examiner

Abstract

In the first chapter, the isomer specific synthesis of polychlorinated dibenzo-p-dioxins, by the pyrolysis of phenolates and the condensation of polychlorinated catechols and polychlorobenzenes, is described. The development of a nitro displacement method and the use of semi-empirical molecular orbital calculations as a means of designing and synthesizing polychlorinated dibenzo-p-dioxins, in moderate to high yield is reported.

The second chapter describes the development of an improved hapten for use in the radioimmunoassay analysis of dioxins. Computational chemistry is used in the identification of the optimum hapten prior to the synthesis of the hapten. The final step, the iodination of the hapten, was not realized in spite of the use of various iodination reagents.

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Acronyms used in this thesis

Ac	acetate or acetyl
ACN	Acetonitrile
ADP	Adenosine 5'-diphosphate
AMP	Adenosine 5'-monophosphate
Ar	aryl
ATP	Adenosine 5'-triphosphate
9-BBN	9-borabicyclo[3.3.1]nonane
BHC	benzene hexachloride
BMS	borane-methylsulfide complex
Bn	benzyl
BSA	N,O-bis(trimethylsilyl)acetamide
BTEAC	benzyltriethylammonium chloride
Bu	Butyl
<i>n</i> -Bu	<i>n</i> -butyl
<i>i</i> -Bu	isobutyl
<i>s</i> -Butyl	<i>sec</i> -butyl
<i>t</i> -butyl	<i>tert</i> -butyl
Bz	benzoyl
CAN	ceric ammonium nitrate
CoA	coenzyme A
Cp	cyclopentadiene
DCC	dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
Dibal-H	Diisobutylaluminum hydride
DME	dimethoxyethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
Et	ethyl
Hex	hexane or hexyl
LAH	lithium aluminum hydride
M	metal
Me	methyl
MEM	methoxyethoxymethyl
MEMCl	methoxyethoxymethyl chloride
MOM	methoxymethyl
MPLC	medium pressure liquid chromatography
Ms	mesyl or methanesulfonyl
MsCl	methanesulfonyl chloride
ND	not detected
NR	not reported
Nu	nucleophile
PCC	pyridinium chlorochromate
PCP	pentachlorophenol
PDC	pyridinium dichromate
PG	protective group
Ph	phenyl
Pr	propyl
ptlc	preparative thin layer chromatography
Pyr	pyridine

RNA	ribonucleic acid
SAA	succinic anhydride
TCP	trichlorophenol
TEA	triethylamine
TEA	triethylamine
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
THP	tetrahydropyran or tetrahydropyranyl
tlc	thin layer chromatography
TMS	trimethylsilyl
TMSCl	trimethylsilyl chloride

Introduction

Four types of chlorinated aromatic compounds have captured the public's interest in recent years: the polychlorinated biphenyls (PCBs, 1), polychlorinated dibenzofurans (PCDFs, 2), the polychlorinated dibenzo-*p*-dioxins (PCDDs, 3) and the chlorinated phenoxyacetic acids (CPAs) (figure 1).

Figure 1

The word 'dioxin' invokes a highly emotional reaction because of the association of dioxin with environmental contamination and its unknown health effects on human beings. The toxic responses displayed by animals exposed to PCDDs (notably 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or 2,3,7,8-TCDD), PCDFs and PCBs include ¹:

- 1) a wasting syndrome
- 2) skin disorders
- 3) lymphoid involution and immunotoxicity
- 4) porphyria
- 5) endocrine and reproductive dysfunction
- 6) teratogenesis: cleft palate and kidney disease
- 7) the induction of diverse enzymes and several hepatic drug metabolizing enzymes and the cytochrome P-448-dependent monooxygenases, aryl hydrocarbon hydroxylase (AHH)
- 8) binding to a cytosolic receptor protein found in diverse tissues

Several recent incidents, outlined below, which have been highly publicized indicate how humans have been exposed to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) or polychlorinated biphenyls (PCBs) ²:

- 1) exposure of U.S. and Australian armed forces personnel and Vietnamese civilians: primarily 2,3,7,8-TCDD which was present as a contaminant in the defoliants used in the Vietnam war. The levels of dioxins in the defoliants used is shown in Table 1.

Table 1

Herbicides used in the Vietnam War ³

Code name	Herbicide*	Quantity (gal)	Period of use	2,3,7,8-TCDD (ppm) [§]
Orange	2,4-D; 2,4,5-T	10,646,000	1965-70	1.98
White	2,4-D; picloram	5,633,000	1965-71	-
Blue	Cacodylic acid	1,150,000	1962-71	-
Purple	2,4-D; 2,4,5-T	145,000	1962-65	32.8
Pink	2,4,5-T	123,000	1962-65	65.6
Green	2,4,5-T	<u>8,200</u>	1962-65	65.6
		17,705,200		

2,4-D = 2,4-dichlorophenoxyacetic acid 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid

[§] estimated total amount of 2,3,7,8-TCDD contained in the Herbicides used was approximately 130 Kg

* 2,4-D and 2,4,5-T were formulated as *n*-butyl esters

-
- 2) occupational exposure of workers to diverse PCDDs and PCDFs in industries which prepare and use chlorinated phenols and derivatives of these products.
 - 3) exposure to 2,3,7,8-TCDD in factories in which chlorinated phenols (2,4,5-T) have reacted to form PCDDs during overheating.
 - 4) exposure to 2,4,5-trichlorophenolate and 2,3,7,8-TCDD as a result of the Seveso incident, 1976.
 - 5) Yusho poisoning in Japan and Taiwan (PCB contaminated rice oil, 1968).

- 6) exposure to PCDFs as a result of PCB fires (Binghamton (NY) state office building, 1981 and St. Basile le Grande, 1988)
- 7) careless disposal of contaminated waste (Times beach, Love Canal, Verona (Missouri) and Gulfport (Mississippi)).

The halogenated dibenzo-*p*-dioxins and dibenzofurans are compounds which are formed as byproducts in the synthesis of chlorinated phenols and PCBs or as byproducts of organic combustion

There are three main sources of PCDDs:

- the use of certain herbicides which are contaminated ,
- Chemical waste,
- incineration.

Polychlorinated dibenzofurans are currently known to be more widespread than PCDDs, and enter the environment from two main sources:

- as a contaminant in PCB formulations;
- as a byproduct of incineration (which includes accidental PCB fires).
- more recently the presence of PCDDs and PCDFs in milk cartons, commercial milk and fish has been reported.¹⁰⁴

The most common disposal method is the high temperature incineration of chlorine containing compounds and PCDD/PCDF contaminated waste oils. But there is public concern over the operation and maintenance of the air pollution control devices and the disposal of the resulting fly-ash.

Recently, the Quebec government has met considerable difficulty in attempting to dispose of the remaining PCB containers from the fire at St-Basile-le-Grande. This indicates the complexity of the disposal problem.

Dioxins and dibenzofurans

The dioxin and dibenzofuran terms actually refer to a family of compounds, which possess the basic structures shown in fig. 2

Figure 2

Each of the substituent positions (indicated by the numbers 1-4 and 6-9) may hold a hydrogen atom or other organic substituents. The only differences between the dioxin isomers is the nature and position of the substituents. The compounds which have received the most attention are the chlorinated dioxins and if we consider only the dioxins, furans and PCBs which possess chlorine and hydrogen, there are 75 possible chlorinated dioxin isomers (Table 2), 135 possible chlorinated dibenzofurans (Table 3) and 209 possible chlorinated biphenyls (Table 4).

Table 2
Table of dioxin isomer distribution

<u>number of chlorines</u>	<u>number of isomers</u>
mono	2
di	10
tri	14
tetra	22
penta	14
hexa	10
hepta	2
<u>octa</u>	<u>1</u>
total	75

Table of Dioxin Isomers

1-monoCDD	4	1,2,3,4-tetraCDD	30	1,2,3,4,6-pentaCDD	52
2-monoCDD	5	1,2,3,6-tetraCDD	31	1,2,3,4,7-pentaCDD	53
1,2-diCDD	6	1,2,3,7-tetraCDD	32	1,2,3,6,7-pentaCDD	54
1,3-diCDD	7	1,2,3,8-tetraCDD	33	1,2,3,6,8-pentaCDD	55
1,4-diCDD	8	1,2,3,9-tetraCDD	34	1,2,3,6,9-pentaCDD	56
1,6-diCDD	9	1,2,4,6-tetraCDD	35	1,2,3,7,8-pentaCDD	57
1,7-diCDD	10	1,2,4,7-tetraCDD	36	1,2,3,7,9-pentaCDD	58
1,8-diCDD	11	1,2,4,8-tetraCDD	37	1,2,3,8,9-pentaCDD	59
1,9-diCDD	12	1,2,4,9-tetraCDD	38	1,2,4,6,7-pentaCDD	60
2,3-diCDD	13	1,2,6,7-tetraCDD	39	1,2,4,6,8-pentaCDD	61
2,7-diCDD	14	1,2,6,8-tetraCDD	40	1,2,4,6,9-pentaCDD	62
2,8-diCDD	15	1,2,6,9-tetraCDD	41	1,2,4,7,8-pentaCDD	63
1,2,3-triCDD	16	1,2,7,8-tetraCDD	42	1,2,4,7,9-pentaCDD	64
1,2,4-triCDD	17	1,2,7,9-tetraCDD	43	1,2,4,8,9-pentaCDD	65
1,2,6-triCDD	18	1,2,8,9-tetraCDD	44	1,2,3,4,6,7-hexaCDD	66
1,2,7-triCDD	19	1,3,6,8-tetraCDD	45	1,2,3,4,6,8-hexaCDD	67
1,2,8-triCDD	20	1,3,6,9-tetraCDD	46	1,2,3,4,6,9-hexaCDD	68
1,2,9-triCDD	21	1,3,7,8-tetraCDD	47	1,2,3,4,7,8-hexaCDD	69
1,3,6-triCDD	22	1,3,7,9-tetraCDD	48	1,2,3,6,7,8-hexaCDD	70
1,3,7-triCDD	23	1,4,6,9-tetraCDD	49	1,2,3,6,7,9-hexaCDD	71
1,3,8-triCDD	24	1,4,7,8-tetraCDD	50	1,2,3,6,8,9-hexaCDD	72
1,3,9-triCDD	25	2,3,7,8-tetraCDD	51	1,2,3,7,8,9-hexaCDD	73
1,4,6-triCDD	26			1,2,4,6,7,9-hexaCDD	74
1,4,7-triCDD	27			1,2,4,6,8,9-hexaCDD	75
2,3,6-triCDD	28			1,2,3,4,6,7,8-heptaCDD	76
2,3,7-triCDD	29			1,2,3,4,6,8,9-heptaCDD	77
				1,2,3,4,6,7,8,9-octaCDD	78

Table 3
Table of dibenzofuran isomer distribution

number of chlorines number of isomers

mono	4
di	16
tri	28
tetra	38
penta	28
hexa	16
hepta	4
<u>octa</u>	<u>1</u>
total	135

It can easily be seen that if substituents other than hydrogen and chlorine are allowed to be present that there are thousands of dioxins and dibenzofurans possible.

Polychlorinated Biphenyls (PCBs)

Table 4

Table of biphenyl isomer distribution

<u>number of chlorines</u>	<u>number of isomers</u>
mono	3
di	12
tri	4
tetra	42
penta	46
hexa	42
hepta	24
octa	12
nona	3
<u>deca</u>	<u>1</u>
total	209

Figure 3

PCBs, or Polychlorinated biphenyls (fig. 3), possess properties which made them ideal compounds for industrial and commercial use⁴:

- PCBs do not decompose under normal conditions,
- PCBs adhere to other materials;
- PCBs are slightly soluble in water, but soluble in oils and in animal fats.

Their commercial uses result from the following properties:

- low degree of degradation over long periods;
- compatibility with other commonly used materials;

- high flash point;
- high dielectric strength (>30kV);
- high resistance to breakdown from internal arcing;
- low vapour pressure;
- low thermal coefficient of expansion;
- low pour point (*ie.* low viscosity at low temperatures);
- low rate of heat release in the event of fire;
- good thermal conductivity;
- good specific heat capacity;
- specific gravity above or below that of water or ice;
- reasonable cost and easily supplied.

PCBs were first manufactured on a commercial scale in 1929⁵. About 635 million kilograms of PCBs were produced in North America before their manufacture was banned in 1977. The major use of PCBs was in fluid for industrial electrical equipment but had additional uses in the following materials - waxes, adhesives, heat exchange fluids, vacuum pump oils, caulking compounds, paints, dedusting agents, hydraulic fluids, printing inks, specialized lubricants, cutting oils, sealants, plastizers, carbonless copying paper.

These same properties have resulted in their becoming environmental contaminants as well as political problems. This has recently been seen in the attempts by the government of Quebec to destroy the remaining barrels of PCBs from the fire at St-Basile-le-Grande. The province of Quebec is unable to ship the barrels to Wales because the workers in England will not unload the barrels.

Chlorinated phenoxy acids

The chlorinated phenoxyacetic acids used most commonly are 4-chloro-2-methylphenoxyacetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Usually the herbicide formulations contain esters of the phenoxy acids.

The industrial preparation of the chlorinated phenoxy acids involves a two-step process, and is shown in scheme 1 and scheme 2⁶.

For MCPA and 2,4-D, the first step involves the chlorination of the phenol or cresol followed by a reaction with chloroacetic acid.

In the case of 2,4,5-T, the first reaction is the hydroxylation of 1,2,4,5-tetrachlorobenzene to the corresponding trichlorinated phenol, which is then reacted with the chloroacetic acid.

Scheme 1

Scheme 2

2,3,7,8-TCDD may be formed during the industrial preparation of 2,4,5-T, when the reaction mixture is heated to 230-260°C but is reported to form at temperatures as low as 180°C⁷. The 2,3,7,8-TCDD problem is associated only with the preparation of 2,4,5-T, however, potential dioxins formed from MCPA and 2,4-D are known to be much less toxic¹¹.

Physical properties of Dioxins

Dioxins possess some intriguing physical properties which result in their persistence in the environment. The low water solubility (Table 5 and 6), vapour pressure (Table 7) and the chemical stability account for the persistence of these materials in the environment:

Table 5

Physical Properties

<u>Compound</u>	<u>Water Solubility (g/L) ⁸</u>	
	<u>20°C</u>	<u>40.0°C</u>
<i>p,p'</i> -DDT	7.7×10^{-6}	
1,3,6,8-TCDD	3.2×10^{-7}	3.9×10^{-7}
octaCDD	0.4×10^{-9}	2.0×10^{-9}
2,3,7,8-TCDD	3.17×10^{-7}	

Table 6

Solubility of 2,3,7,8-TCDD in Organic Solvents at 25°C (g/L) ⁹

<u>Solvent</u>	<u>g/L</u>
<i>o</i> -dichlorobenzene	1.400
chlorobenzene	0.720
benzene	0.570
chloroform	0.370
<i>n</i> -Octanol	0.048
methanol	0.010
acetone	0.100

Table 7

Vapour Pressure ⁹

<u>Compound</u>	<u>P(atm)</u>		
	<u>20°C</u>	<u>50°C</u>	<u>100°C</u>
<i>p,p'</i> -DDT	1.7×10^{-10}	6.2×10^{-10}	8.8×10^{-10}
1,3,7,8-TCDD	5.3×10^{-9}	1.3×10^{-8}	1.1×10^{-8}
octaCDD	8.6×10^{-11}	1.1×10^{-10}	1.7×10^{-10}
2,3,7,8- TCDD			1.37×10^{-7}

The low solubility in water accounts for the immobility in most soils. For example, the mobility of 2,3,7,8-TCDD in soils was limited to the upper 8 cm one year after the accident in Seveso, Italy¹⁰.

A number of options have been proposed for the disposal of these materials. These methods include³:

- 1) stabilization in place
- 2) onsite storage
- 3) transportation to secure landfill
- 4) solvent extraction
- 5) biodegradation
- 6) chemical degradation in place (ozonolysis, chlorinolysis, wet oxidation, catalytic dechlorination)
- 7) incineration (the Dow Chemical Plaquemane Incinerator uses an initial incinerator at 1800°F followed by a second incineration of the gases produced at 2400°F)
- 8) photolysis (see scheme 3)
- 9) molten salt combustion
- 10) microwave plasma destruction

Photolytic cascade of 2,3,7,8-TCDD in a hydrogen atmosphere

Scheme 3

Toxicological Studies

The major problems with the toxicological assessment of dioxins and furans is the large species variability for the same dioxin (this difference is about 1000X between the Guinea Pig and the Hamster for 2,3,7,8-tetraCDD ¹¹, Table 8) and the large difference between the dioxins within the same species (eg. for the Guinea Pig the most toxic is 2,3,7,8-tetraCDD with an LD₅₀ of 0.6-2 µg/Kg compared to 1,3,6,8-tetraCDD with an LD₅₀ of >15,000,000 µg/Kg).

Table 8

Comparative Single Dose LD₅₀ Values for Chlorodibenzo-*p*-dioxin Isomers ¹¹

<u>Oral LD₅₀ Values (µg/kg)</u>			
<u>Chlorodibenzo-<i>p</i>-dioxin</u>	<u>Guinea Pig</u>	<u>Mouse</u>	<u>Rat</u>
2,3,7,8-Tetra	0.6-2	114-284	22-45
unsub		>50,000	>1,000,000
2,3-di			>1,000,000
2,7-di		>2,000,000	>1,000,000
2,8-di	>300,000	847,000,000	>5,000,000
1,3,7-tri		>15,000,000	>5,000,000
2,3,7-tri	29,444	>3,000	>1,000,000
1,2,3,4-tetra			>1,000,000
1,3,6,8-tetra	>15,000,000	>2,987,000	>10,000,000
1,2,3,7,8-penta	3.1	337.5	
1,2,4,7,8-penta	1,125	>5,000	
1,2,3,4,7,8-hexa	72.5	825	
1,2,3,6,7,8-hexa	70-100	1250	
1,2,3,7,8,9-hexa	60-100	>1440	
1,2,3,4,6,7,8-hepta	>600		
octa		>4,000,000	>1,000,000
 <u>Chlorodibenzofuran</u>			
2,8-di		>15,000,000	>15,000,000
2,4,8-tri		>15,000,000	>5,000,000
2,3,7,8-tetra	5-10	>6,000	>1,000
2,3,4,7,8-penta	<10		
2,3,4,6,7,8-hexa	120		

Recent data ¹² on dioxins, furans and PCBs residues in birds in the Great Lakes indicates that the birds have ppt levels of dioxins and furans as well as ppm levels of PCBs.

There are 5 to 6 orders of magnitude higher concentrations of PCBs than the levels of dioxin and furans which is the major difficulty in assessing the toxicity in humans and animals because one is not exposed to a single compound in the environment but a complex mixture.

There are a number of factors which effect toxicity; for example, species variability, response differences, and synergistic effects.

Species variability

The guinea pig is very susceptible to 2,3,7,8-TCDD.

A dose of 0.6 - 2.0 μ g / Kg body weight of 2,3,7,8-TCDD will kill a guinea pig, however, the hamster and frog are 1000X less susceptible.

Dose differences

The response of species to low concentrations of dioxins and dibenzofurans is very different than seen at high concentrations.

There are also questions about the long term effects of low doses (ppt) on humans and despite several years of intense study, the precise mechanism by which TCDD and related halogenated compounds cause toxicity remains unclear.

Synergistic effects

A dose of TCDD (20 μ g / Kg) that gave about 60% fetal cleft palate in mice which is a characteristic toxic response when used in a ratio of 10,000 parts of Aroclor 1254 (a commercial PCB) to one part TCDD (20 μ g / Kg) one can almost completely eliminate the cleft palate in the mice. This indicates that the mice received protection from the dioxin by the presence of the PCBs.

Toxicity and Mechanism of Action

Experiments in laboratory animals ¹³ indicate that binding to the *Ah* receptor is a key step in the toxicity of halogenated xenobiotics. Ligands initially bind to the *Ah* receptor in the cytoplasm (fig 4), after which the ligand-receptor complex trans-locates into the nucleus in a temperature-dependent step.

Figure 4 ¹³

The *Ah* receptor is a soluble cytoplasmic protein with a molecular weight greater than 200,000. The cytoplasmic *Ah* receptor, a product of the regulatory gene in the *Ah* gene complex, binds TCDD as well as non-halogenated polycyclic aromatic hydrocarbons of the "3-methylcholanthrene-type". After "activation" and translocation into the nucleus the receptor ligand complex stimulates synthesis of mRNAs which code for cytochrome P₁-450 and other structural parts associated with the *Ah* gene complex.

P₁-450 induction *via* the *Ah* receptor is important in increasing conversion of many xenobiotic chemicals into chemically reactive intermediates that bind covalently to macromol-

ecules, thereby leading to cytotoxicity, mutations or cancer. Although the toxicity of TCDD and related halogenated compounds appears to be mediated through the *Ah* receptor, this toxicity does not require P₁-450 induction nor metabolism of TCDD. It is not known which of the other cellular events regulated by the *Ah* receptor are responsible for the toxic responses to halogenated compounds such as TCDD¹⁶.

A number of studies in recent years have shown similarities between the action of TCDD on cells and the effects of glucocorticoid hormones¹⁴. McConkey *et al*¹⁴ found that 2,3,7,8-TCDD treatment leads to an increase in Ca²⁺ in the cytoplasm of lymphocytes from rat thymus, followed by fragmentation of the cells' DNA, then cell death. The calcium ion controls processes ranging from muscle contractions to cell division¹⁵.

The effect of calcium is to speed up the metabolic processes that supply the respiratory chain thereby increasing the production of ATP. This chain of events may serve as a feedback mechanism that helps the cell defend itself against excessive levels of calcium ion. When the cell is challenged by very high intracellular calcium, it needs to operate all its calcium removal mechanisms. All of them require energy generated by the respiratory chains. The two calcium-transporting ATPases use ATP directly. Thus, the increase in calcium concentration will remove ATP which is necessary for the continued growth of the cell.

Brownlee *et al*^{16a-d} have studied the mixed function oxidase (MFO) response to the physico-chemical properties of a xenobiotic. The authors noted that the specific activity *in vitro* was not dependent on the substrate structure but was inversely related to the weakest C-H bond strength of the substrate. For the dioxins studied, they determined that two mechanisms may be involved:

C-Cl bond metabolism, occurring most easily in fully chlorinated rings, and C-H bond metabolism.

Xenobiotics act in three distinct fashions when introduced in to biological systems:

1) **Promoter Behaviour** - The xenobiotic induced the production of large amounts of powerful enzymes to degrade the xenobiotic and after the removal of the xenobiotic the remaining enzymes work on any materials which are available to the enzyme.

2) **Agent Provocator** - Very strong C - H bonds that cannot be hydrolysed (even if the enzyme system is modified) or the xenobiotic is kinetically protected (if the carbon possesses a large positive charge, the enzyme cannot approach the molecule) the xenobiotic is not hydrolysed. The presence of the molecule leads to the increased production of enzymes and this exhibits itself by the increased concentration of P-450 enzymes, leading to a condition known as porphyria.

3) **Suicidal** - The xenobiotic is oxidized by the enzyme and very little of the enzyme remains after the reaction. Most of the enzyme is rapidly denatured.

If the induction is directed towards C - H bond strengths in the MFO (mixed functional oxidases) systems of skin, lung, intestine, liver, and kidney, then the enzymes which have been induced produce increased hydrogen peroxide in the cytosol and, accidentally, highly oxidizing free radicals from the substrate in the lipids. The rapidly reacting radicals can attack the heme site, the surrounding protein, or the lipid, generating other radicals. The destruction may not terminate locally, but can be detected as free-radical alkylation processes in the cytosol and nucleus leading to mutagenesis and possibly carcinogenesis.

The calculation of the C - H bond strength of some xenobiotics and the local charges will be discussed later in conjunction with some theoretical calculations which are currently being investigated.

Any attempt at risk assessment will require further data using pure compounds and with mixtures to test for antagonistic effects. As was reported above Aroclor 1254 has been shown to act as an antagonist towards 2,3,7,8-tetraCDD ¹².

Analytical Analysis

In the area of detection, it is known that all dioxins do not react in the same way to the different detection systems (ie. GC. MS, ECD, RIA). It is necessary to obtain pure standards of the dioxins to standardize the testing systems and radiochemical standards for use as surrogates. All of these questions will require the use of pure analytical and toxicological standards to further our understanding of these and related compounds.

Synthesis of dioxins

There are two reported methods to produce dioxins, the first method is the phenolate coupling reaction which involves the pyrolysis of chlorinated phenolate salts in the presence of copper metal ¹⁷. The second method involves the reaction of catechol (1,2-dihydroxybenzene) with a chlorinated benzene ^{18,19} (see fig. 5).

Figure 5

Of the available methods to produce dioxins, the method of Gray, Cepa, Solomon and Aniline¹⁹ seems to give the greatest control over the production of specific dioxins. This method involves the reaction of a suitably substituted catechol **76** (1,2-dihydroxybenzene) with a substituted polychloro- (or polychloronitro-) benzene **77** (fig. 6).

Figure 6

This method was found to be superior in the production of dioxins which were sensitive to the pyrolysis conditions of the first method using two suitable substituted chlorinated phenols (see fig.7).

Figure 7

The major problem associated with the phenolate method for the production of chlorinated dioxins is the subsequent loss of chlorines from the final product. This loss of chlorines is a direct result of the harsh conditions used to produce the desired materials and results in the formation of complex mixtures. This problem is illustrated in the analysis of commercial pentachlorophenol and its sodium salt by Buser et al ²⁰. The fragmentogram[†] (fig 8) of these samples indicated the presence of not only octachlorodibenzo-*p*-dioxin but also hepta-, hexachlorinated dibenzo-*p*-dioxins (DD) as well. Also noted in some of the samples were some tetrachlorinated DD.

Figure 8

The pyrolysis of 2,3,6-trichlorophenol indicates another problem which is associated with this method ²¹. If there are two possible sites which may react to form a dioxin. This

[†] A fragmentogram is produced by using a mass spectrometer as the detector on a gas chromatograph. This yields not only the intensity of the peak but the mass spectrum of the peak as well.

Figure 9

reaction results in the formation of four products which must be separated (fig 9). Another problem associated with the pyrolysis route is the formation of complex mixtures of the dioxins which result from the use of different phenols. If two phenols, A and B (ie. in fig. 7 x is not the same as y), are allowed to react one would produce not only the addition products to form C, D and E but one would also obtain from three to six products arising from the Smiles rearrangements (more will be produced if there are multiple sites of attack on the phenol).

Figure 10

There are two possible products from the reaction of phenols A and B, this second product results from a further complication in the reaction because the intermediate formed during the initial phase of the condensation is able to undergo a Smiles rearrangement (see fig.10) and see p. 21.³⁰

This rearrangement results in the formation of two compounds, in all cases of the synthesis of dioxins (by any means), except when the compound is symmetrical. The problem of the separation of the two dioxins produced by the condensation of the catechol with the nitrochlorobenzene is not a trivial problem because the isomeric dioxins were found to possess only slight differences in their chromatographic properties ^{21a} (Table 9). It is not possible, even now, to separate all of the tetrachlorodibenzo-*p*-dioxins by gas chromatography (GC).

Table 9
TCDD retention times on Reverse-phase and Silica Gel

TCDD isomer	RP-HPLC abs RT min	Silica HPLC rel RT
1,2,6,7/8,9	12.2-12.9	1.623
	12.2-12.9	1.795
1,2,6,8/7,9	13.3-13.9	1.238
	13.3-13.9	1.291
1,2,6,9	11.6-13.0	1.702
1,2,7,8	14.0-14.7	1.288
1,3,6,8	15.9-16.8	0.977
1,3,6,9/1,4,7,8	13.3-13.9	1.220
	13.3-13.9	1.340
1,3,7,8	14.9-15.7	1.000
1,3,7,9	14.9-15.9	0.940
1,4,6,9	11.6-13.0	1.497
2,3,7,8	13.8-14.5	1.000
1,2,3,6/3,9	13.8-14.4	1.356
	13.8-14.4	1.350
1,2,3,7/3,8	14.0-15.0	1.100
	14.0-15.0	1.128
1,2,4,6/4,9	13.7-14.5	1.328
	13.7-14.5	1.441
1,2,4,7/4,8	14.2-15.1	1.154
	14.2-15.1	1.199
1,2,3,4	15.8-16.8	1.248

(rel RT = as computed from absolute retention time of 2,3,7,8-TCDD = 1.000 $\pm$ 0.01)

Recently, a new column has been prepared which can separate 20 of the 22 tetrachloro-dibenzo-*p*-dioxin isomers.^{21b,c}

Results and Discussion

Chapter 1

Synthesis of Dioxins as Analytical and Toxicological Standards

Dioxin (PCDD) Synthesis *via* the Pyrolysis of Chlorophenoxides

The synthesis of dioxins *via* the phenolate method was performed following the standard method of phenolate pyrolysis (*phenolate is an old term which is equivalent to phenoxide and will be used in this thesis for historical clarity*)²²⁻²⁶. The dioxins which have been synthesised by this method are indicated in Table 10. In most of the cases described, the synthesis resulted in the formation of more than one product.

TABLE 10

Dioxins (PCDD) synthesized *via* the Phenolate Method

<u>Chlorophenol(s)</u>	<u>Dioxin(s) produced</u>	<u>Yield</u>	<u>Ref</u>
2-chloro	unsub	25%	23
2-chloro + PCP	1234	-	23
2,5-dichloro	2,7	-	23
2,4-dichloro + 2,3,4,5-tetrachloro	1237	-	21
	1238		
2,5-dichloro + 2,3,4,5-tetrachloro	1237	-	21
	1238		
2,6-dichloro + 2,3,4,6-tetrachloro	1236	-	21
	1239		
2,5-dichloro + 2,3,4,6-tetrachloro	1237	-	21
	1238		
	1247		
	1248		
2,4-dichloro + 2,3,5,6-tetrachloro	1247	-	21
	1248		
2,5-dichloro + 2,3,5,6-tetrachloro	1247	-	21
	1248		
2,3,4-trichloro	1267	-	21
	1289		
2,3,5-trichloro	1368	-	21
	1379		
2,3,6-trichloro	1267	-	21
	1269		
	1289		
	1469		
2,4,5-trichloro	2378	-	21
2,4,6-trichloro	1368	-	21
	1379		
2,3,4-trichloro + 2,3,5-trichloro	1268	-	21
	1279		
2,3,4-trichloro + 2,3,6-trichloro	1267	-	21
	1269		
	1289		
2,3,4-trichloro + 2,4,5-trichloro	1278	-	21
2,3,5-trichloro + 2,3,6-trichloro	1268	-	21
	1279		
	1369		

TABLE 10 continued
Dioxins synthesised *via* the Phenolate Method

2,3,5-trichloro + 2,4,5-trichloro	1378	-	21
2,3,6-trichloro + 2,4,5-trichloro	1278	-	21
	1278		
2,3,4-trichloro + PCP	1267	-	23
	123467		
	octa		
2,3,5-trichloro + PCP	1368	-	23
	123468		
	octa		
2,3,6-trichloro + PCP	1267	-	23
	1269		
	1469		
	123467		
	123469		
2,4,5-trichloro + PCP	2378	-	23
	123478		
	octa		
2,3,4,5-tetrachloro	123678	-	23
	123789		
2,3,4,6-tetrachloro	123689	-	23
	123789		
	124679		
2,3,5,6-tetrachloro	124679	-	23
2,3,4,5-tetrachloro + 2,3,5,6-tetrachloro	123678	-	23
	123689		
	123789		
	124679		
2,3,4,5-tetrachloro + PCP	123678	-	23
	123789		
	1234678		
	octa		
2,3,4,6-tetrachloro + PCP	123678	-	23
	123689		
	123789		
	124679		
	1234678		
	1234679		
	octa		
2,3,5,6-tetrachloro + PCP	124679	-	23
	1234679		
	octa		
PCP	octa	4%	23

PCP = 2,3,4,5,6-pentachlorophenol

The reaction requires the formation of the potassium salt of the chlorophenol; usually by the addition of the chlorophenol to a potassium hydroxide solution or methanolic potassium hydroxide. The copper metal (electrolytic copper dust) was added to the phenolate salt solution and with mixing, the solvent was removed under a stream of argon and then the phenolate salt was placed under vacuum and then slowly heated in a vacuum sublimation apparatus to a temperature between 150°C to 320°C, at ~10-12 mmHg.

One of the major problems involved in the pyrolysis of phenolates is the Smiles rearrangement and this problem is observed in the pyrolysis of 2,3,6-trichlorophenol **86**. The reaction pathway for the production of the four dioxins (1,2,6,7-TCDD **39**, 1,2,6,9-TCDD **41**, 1,2,8,9-TCDD **44** and 1,4,6,9-TCDD **49**) which result from the pyrolysis of 2,3,6-trichlorophenol **86** is shown in scheme 4.

Scheme 4

The identification of the dioxin isomers was accomplished using the method of Buser and Rappe (see page 31).

Preparation of dioxins in this study *via* the pyrolysis of phenols

An example of the synthesis of dioxins is the pyrolysis of the potassium phenolate of 2,3,6-trichlorophenol **86a**, which was mixed with 1 eq. of Cu⁺ (electrolytic dust) and placed in a sublimation apparatus. The system pressure was reduced to 10 mmHg and then the temperature was slowly increased over 30 minutes to 170°C and the temperature was maintained at 170°C for five hours. The temperature was then allowed to drop to room temperature and the vacuum was reduced by the introduction of an overpressure of argon. The sublimate was removed from the cold finger and the tlc (CCl₄) indicated the presence of some starting phenol. After purification, by ptlc (CCl₄) to remove the small amount of starting phenol, the GC/MS (fig. 11) indicated the presence of two tetrachlorodibenzo-*p*-dioxins and four trichlorodibenzo-*p*-dioxins and a "predioxin". The two tetraCDDs, labeled F and G in fig. 11, structures have been assigned as 1,4,6,9-TCDD **49** and 1,2,6,9-TCDD **41**, respectively. The peak at E, according to the MS was assigned the structure of a predioxin (fig. 11).[†]

Figure 11

[†]The trichloroDDs are formed during the synthesis and not due to loss of chlorine during the GC / MS. The use of helium as the carrier inhibits the loss of chlorine during the GC run.

The formation of the trichlorinated dioxins indicates the second problem associated with the phenolate method, the loss of chlorines during the pyrolysis.

The pyrolysis of 2,3,4-trichlorophenol **89** at 200°C produced a mixture of 1,2,6,7- and 1,2,8,9-TCDD, **39** and **44** respectively, in high purity, but low chemical yield (2.4%). The identities of the dioxins were confirmed by GC/MS and by comparison to standards. In the pyrolysis of 2,3,5,6-tetrachlorophenol **94**, no reaction occurred when the pyrolysis was performed at 160°C, only starting material was obtained. The reaction temperature was increased to 200°C for one hour then the reaction temperature was increased to 250°C and was maintained at 250°C for twelve hours. The sublimate obtained was purified on ptlc (CCl₄) to remove the small amount of starting phenol in the sublimate. The GC/MS indicated only one hexa-isomer and a small amount of pentaCDD and tetraCDD, in the sample, but the chemical yield was low (2.3%).

The reaction was repeated, but the initial temperature used was 250°C and the reaction temperature was increased to 320°C over thirty minutes and was maintained at 320°C for three hours. The sublimate was purified, after cooling, by ptlc (CCl₄), to yield the hexachlorodioxins **74** and **75**, in 10% yield.

The pyrolysis of 2-chlorophenol **88** at 250°C for two hours, after which, the solid sublimate (needles) were removed from the cold finger and tlc indicated the presence of only unsubstituted dioxin. GC/MS confirmed the identity as dibenzo-*p*-dioxin. The dioxins prepared in this laboratory are shown in Table 11.

Table 11
Dioxins produced by the phenolate method.

<u>phenol</u>	<u>dioxin(s)</u>	<u>chemical yield</u>
2-chlorophenol	dioxin	45%
2,3,4-trichlorophenol	1,2,6,7/8,9	2.4%
2,3,6-trichlorophenol	1,2,6,9 and 1,4,6,9	3.5%
2,3,5,6-tetrachlorophenol	1,2,4,6,7,9/6,8,9	10%

Identification of Dioxins by GC / MS

Buser and Rappe²⁷ found that the identification of discrete isomers was difficult using normal techniques. Techniques other than mass spectroscopy use large amounts of sample and normally require the use of pure standards to calibrate the detection system.

The electron-impact (EI) mass spectra allows an easy identification of PCDDs and in distinguishing them from other polychlorinated compounds such as dibenzofurans, diphenyl ethers, biphenyls and naphthalenes^{28,29}.

The identification is based on the intense molecular ions (M^+) with the expected clustering due to the chlorine isotopes (fig12) and the characteristic fragmentation with the formation of $M^+ - \text{COCl}$ and $M^+ - 2\text{COCl}$.

Figure 12

The authors reported a general approach that allows the identification of a substitution pattern of a PCDD by observation of its lower mass ions.

As the substitution pattern, the authors defined this as the number of chlorine atoms in each carbon ring of a dioxin molecule.

Table 12

# of Isomers and Substitution pattern of PCDDs²⁷				
Compound	# chlorine in each ring		# of isomer	Total # of isomers
	X	Y		
mono-CDD	1	0	2	2
di-CDD	2	0	4	10
	1	1	6	
tri-CDD	3	0	2	14
	2	1	12	
tetra-CDD	4	0	1	22
	3	1	8	
	2	2	13	
penta-CDD	4	1	2	14
	3	2	12	
hexa-CDD	4	2	4	10
	3	3	6	
hepta-CDD	4	3	2	2
octa-CDD	4	4	1	1
Total				75

This table²⁷ indicates that the number of positional isomers of PCDD can be subdivided into smaller groups.

The authors found that there were key low mass ions of PCDDs (Table 13) which are indicative of the chlorinated carbon rings and allow a determination of the chlorine substitution pattern of an individual PCDD isomer.

Table 13

**Characteristic Ions in the mass spectrum which are indicative of the
different chlorinated carbon rings of PCDDs**

<u>#of Chlorines</u>	<u>m/e Values (composition of some characteristic ions)</u>		
0	76 (C ₆ H ₄ ⁺) 52 (C ₄ H ₄ ⁺)		
1	75 (C ₆ H ₃ ⁺) 51 (C ₄ H ₃ ⁺)	110 (C ₆ H ₃ Cl ⁺)	
2	<u>74</u> (C ₆ H ₂ ⁺) 50 (C ₄ H ₂ ⁺)	109 (C ₆ H ₂ Cl ⁺) 97 (C ₅ H ₂ Cl ⁺) 85 (C ₄ H ₂ Cl ⁺)	
3		<u>108</u> (C ₆ HCl ⁺) 96 (C ₅ HCl ⁺) 84 (C ₄ HCl ⁺)	143 (C ₆ HCl ₂ ⁺) 131 (C ₅ HCl ₂ ⁺)
4		107 (C ₆ Cl ⁺) 95 (C ₅ Cl ⁺)	<u>142</u> (C ₆ Cl ₂ ⁺) 130 (C ₅ Cl ₂ ⁺) 118 (C ₄ Cl ⁺)

major ions are underlined

The Smiles Rearrangement³⁰

The first report of a Smiles rearrangement was in 1894 when Henriques reported that bis-(2-hydroxy-1-naphthyl) sulfide was converted to an isomeric dibasic substance by treatment with alkaline ferricyanide and subsequent reduction of the intermediate obtained (fig. 13)³¹.

Figure 13

The rearrangement may be represented in overall form as follows. The two carbon atoms joining groups X and Y may be aliphatic or part of an aromatic ring; in a few cases hetero atoms have replaced one of these carbon atoms (fig. 14).

Figure 14

Several pathways are possible for the conversion of 105 to 106; for example, the nucleophilic function, YH, may be ionized with substitution occurring *via* the transition state

107 (fig. 15).

Figure 15

On the otherhand, prior ionization is not always required, and the rearrangement may proceed in a more concerted fashion through transition state 108 (fig. 16). There is also the possibility that in certain systems the rearrangement proceeds through a stabilized intermediate such as 109, as opposed to a transition state (fig. 17).

Figure 16

Figure 17

Most Smiles rearrangements require electronic activation in the migrating aromatic ring. The most common activation group is a nitro group in the *ortho* or *para* position, with or without additional substituents³².

Galbraith and Smiles³³ made an extensive study of the effect of activation on the rate of rearrangement of typical compounds prepared in Smiles' studies.

They observed the rearrangement of a number of sulfones of types **110** and **111** (fig. 18). The following order of reactivity was observed:

When R was a second electron-withdrawing substituent, the compounds of type **110** reacted faster than those of type **111** and the nitro group is more effective in the 2-position than in the 4-position.

Smile's³⁴ studies showed that, if X is a good leaving group, or if Y⁻ is a strong nucleo-

110

111

Figure 18

phile, rearrangement occurs readily. As X becomes less replaceable or as Y^- becomes a weaker nucleophile, rearrangement is more difficult.

The acidity of the YH function was determined by the use of various bases in the corresponding hydroxylic solvents, the rate of rearrangement decreased in the order:

This is also the order observed for the basicity of these reagents.

The acidity of the YH function is not always an independent factor in the rearrangement, since substituents which enhance the acidity of the group YH may decrease the nucleophilicity of the anion Y^- . The net effect on the rearrangement must be the resultant of these two counterbalancing effects.

The effect of substituents in the aromatic ring joining X and Y are much more complex than those in the ring sustaining the nucleophilic attack. Most of the systems studied were of the structural type 112 (fig. 19).

112

Figure 19

It is readily seen that any substituent in ring A will be able to influence the reaction both by its effect on the group YH and the corresponding anion, Y^- , and, also by its effect on the transition state leading to the rearranged product.

Thus an electron-withdrawing substituent (Table 14) at positions 4 or 6 may by induction, increase the acidity of the group YH and may, by induction or resonance, stabilize the charge appearing on X as the reaction proceeds.

Table 14**Table of Electron donating and Withdrawing groups^{36,37}**

<u>Electron donating</u>		<u>Electron-Withdrawing</u>	
O ⁻	SR	NO ₂	CHO
S ⁻	SH	CN	COR
NR ₂	Br	COOH	SO ₂ R
NHR	I	COOR	SO ₂ OR
NH ₂	Cl	CONH ₂	NO
NHCOR	F	CONHR	Ar
OR	R	CONR ₂	
OH	H		
OCOR			

These effects appear to be favorable for rearrangement unless the group YH is sufficiently acidic to be able to ionize readily. Therefore the effect of an electron-withdrawing substituent at the 4-position or any other position in the ring may serve to reduce the nucleophilicity of the anion, Y^- , to the detriment of the rearrangement. Electron-donating

groups would have the opposite effect (fig. 20).

4 or 6 NO_2 , NR_3 , Cl , etc.

3 or 5 O^- , COO^- , CR_3

4 and 6 --- electron-withdrawing groups aid displacement

5 ----- electron-donating groups aid displacement

Figure 20

The Nitro Group Effect In Aromatic Displacement

Gray *et al*³⁸ reported that both the Ullmann copper-catalyzed self-condensation of the appropriate potassium phenolates and catechol condensation reactions proceed *via* Smiles rearrangement. Evidence suggests that a Smiles rearrangement intermediate always intervenes in these processes and that the proportion of isomers formed may be thermodynamically controlled.

Gray *et al*³⁸ reported that in the self-condensation of the potassium salt of 2-bromo-3,4,5-trichlorophenol **114** (fig 21) the initially formed anion **115A** rapidly equilibrates through **116** with the rearranged anion **115B**. Then, slowly **115A** cyclizes to yield **70** and **115B** cyclizes to yield **73**.

Figure 21

The intermediacy of the Smiles rearrangement was shown by Kende and DeComp³⁹ when they attempted the regiospecific synthesis of **70** and **73**. The reaction of the potassium salt of 4,5,6-trichloroguaicol **117** with 2,3,4,5-tetrachloronitrobenzene **118** gave about a 1:1 mixture of the two diphenyl ethers **119A** and **B** (fig 22).

Figure 22

The similar reaction of 3,4,5-trichloroguaiacol **120** with 2,3,4,5-tetrachloronitrobenzene **118** gave a mixture of the two diphenyl ethers **121A** and **B** (fig 23).

Figure 23

The treatment of **119A** and **B** or **121A** and **B** with LiI in DMF or DMSO effected both the removal of the methyl protecting group and the intramolecular cyclization of the pre-dioxin to yield the hexachlorodioxin isomers **70** and **73** and small amounts of nitropoly-chlorodibenzo-*p*-dioxins **122** (fig. 24).

Figure 24

The authors stated that the formation of both hexachlorodioxin isomers, in the same ratio, from the precursors **119A** and **B** and **121A** and **B** requires the rapid interconversion between the two phenoxides **123** and **124**. They proposed that the interconversion of the two phenoxides is accomplished *via* the Smiles rearrangement intermediate **125** (fig. 25).

Figure 25

Synthesis of chlorinated catechols

The 9 chlorinated catechols (fig 26) required for the synthesis of all polychlorinated dioxins have commonly been prepared by one of the two methods shown in fig 27.

Figure 26

Figure 27

Method 1

Method 1 involves the direct chlorination of catechols using the method of Willstater and Müller ⁴⁰. This method is useful in the synthesis of 4-chlorocatechol **127**, 4,5-dichlorocatechol **129** and 3,4,5,6-tetrachlorocatechol **134** (Table 15). This method is not synthetically useful for the synthesis of 3,4,5-trichlorocatechol **133** because once the third chlorine is attached, the trichlorocatechol is rapidly attacked by a fourth chlorine and the tetrachlorocatechol **134** is produced.

The catechols synthesis *via* this method are indicated in Table 15.

Table 15

Catechols produced *via* direct chlorination (Method 1)¹⁹

<u>Catechol</u>	<u>% yield</u>
4-chloro	33%
4,5-dichloro	24%
3,4,5-trichloro	6%
3,4,5,6-tetrachloro	7%

Method 2

The second method involves the production of a salicylaldehyde as an intermediate and the subsequent conversion of the formyl group to a hydroxyl group. The salicylaldehyde may be produced by two methods, the Reimer-Tiemann or the Duff reactions ⁴¹ (fig 28).

Figure 28

The first method, discovered by Reimer and Tiemann^{42,43}, suffers from the formation of complicated mixtures, the *ortho*- and *para*- aldehydes, products containing chlorine or ketone groups⁴⁴.

The preferred method is *via* the Duff reaction,^{41,45} because this method yields only *ortho*- substitution, or small amounts of *ortho*- *para*- dialdehydes. The yields are lower (15 - 20%) but the product is much purer and takes less time to prepare.

The next step requires the conversion of the resultant salicylaldehyde to the desired catechol using the classical Dakin reaction⁴⁶ (fig. 28).

In the oxidation of the 3,5-dichlorosalicylaldehyde **135** (fig. 28) with hydrogen perox-

ide, the yield of 3,5-dichlorocatechol **130** was greatly improved by constantly degassing the reaction with Argon. Without degassing, the yield of the reaction was only 3% but with constant degassing the yield, in this study, was increased to 50%.

It is possible to convert, the methyl group of trinitrotoluene, into a hydroxyl group by the reaction of potassium dichromate, sulfuric or nitric acids ⁴⁷. The reaction involves the following steps:

- 1) the oxidation of the methyl group to the corresponding acid,
- 2) nucleophilic substitution of the COOH group by NO_3^- ,
- 3) hydrolysis of the nitrate ester.

However, these conditions would be too severe for the catechols to survive. It would be necessary to use protecting groups which would prove difficult to remove after the conversion.

Sato *et al* ⁴⁸, reported the conversion of cyclohexanones into their corresponding mono-, di-, or trichlorocyclohexenones by the reaction with cupric chloride (fig 29).

Figure 29

By reacting 2-chlorocyclohexanone **136**, in 50% dioxane / water with cupric chloride dihydrate, only 3-chloro-2-hydroxycyclohexen-1-one **137** was isolated in 62% chemical yield (lit. yield 82% from cyclohexanone). By increasing the concentration of cupric chloride, it was possible to produce the di- and trichlorocyclo-2-hydroxycyclohexen-1-ones **138** and **139**, but the separation of di- and trichloro species was very difficult.

The yield and selectivity of the product was improved by changing the solvent to 50%

Acetic acid. By changing the solvent, it is possible to selectively produce; in moderate yield, the di- and trichloro-2-hydroxycyclohexen-1-one, by altering the amount of cupric chloride (20% eq. for dichloro and 30% eq. for trichloro) and in high purity.

The authors reported that the mechanism for the reaction was shown in scheme 5:

Scheme 5

It has previously been reported that an α -chlorination easily occurs *via* the enol complex in the reactions of saturated ketones with copper(II) chloride. The reaction is initiated by an attack on the α -carbon in cyclohexanone with copper(II) chloride, followed by 2, 2- and / or 2, 6-dichlorination.

The α -monochlorination **136** product is chlorinated to give a dichloroproduct (2, 2-dichloro **140** and / or 2, 6-dichloro **141**) which is quickly chlorinated to give the 2, 2, 6-

trichlorocyclohexanone **142**. The trichlorinated cyclohexanone is then hydrolysed to give 3-chloro-2-hydroxycyclohex-2-en-1-one **137**. The monochlorinated species **137**, is further chlorinated at the α -position with respect to the carbonyl to give the di- and trichloro species **138** and **139** respectively.

By limiting the amount of cupric chloride, the authors proved the intermediacy of **137** to **138**, by forming these products selectively.

Synthesis of 3-chloro- and 3,6-dichlorocatechol via the method of Satoh *et al*⁴⁹

Of the three compounds which could be produced, only two (the di- and tri-chloro species) are synthetically useful. Satoh *et al*⁴⁹ reported that the reaction of the di- and tri-chloro-2-hydroxycyclohex-2-en-1-one **138** and **139** with lithium carbonate, in dimethyl formamide (fig 30) gave the corresponding chlorinated catechols in low yield (3-chlorocatechol **128** - 26%, 3,6-dichlorocatechol **131** - 28%) but high purity. The 3-chlorocatechol and 3,6-dichlorocatechol were found to readily crystallize, after purification on the chromatotron (silica 2mm, 10% ether / toluene to 40% ether / toluene gradient).

Figure 30

Because of the low yields obtained by the method of Satoh *et al*⁴⁹ in the synthesis of 3-chlorocatechol and the work of Sniekus *et al*⁵⁰⁻⁵⁵, Meyers *et al*⁵⁶ and Posner *et al*⁵⁷, who have reported on the *ortho*-lithiation of aromatics it was decided to attempt an *ortho*-lithiation reaction, to produce the 3-chlorocatechol **128**. Christensen⁵⁸ reported the synthesis of 3-chlorosalicylaldehyde **145**, by the *ortho*-lithiation of the appropriate phenyl ethers and subsequent reaction with dimethylformamide. The formyl group was introduced into the *ortho*-position by taking advantage of the fact that the methoxymethyl ether can be lithiated exclusively *ortho* to the oxygen atom (fig. 31).

Figure 31

Mechanism of the *ortho*-lithiation ^{59a}

Shirley *et al* ⁵³ has shown that moderate to high selectivity can be obtained in the metalation of phenyl ethers (fig. 32). The selectivity of 1,3-dimethoxybenzene **146** and ethyl phenyl ether **147** has been found empirically to metalate in the *ortho*- position in >96%

Figure 32

yield.

Two different interpretations of the mechanism of the metalation reaction have been advanced ⁵⁹⁻⁶⁰.

Mechanism I

The organometallic ion-pair is pictured as forming a coordinate bond between the metal cation and a pair of electrons belonging to a hetero atom (eg. halogen, oxygen, nitro-

Figure 33

gen, etc.) of the molecule undergoing metalation (fig 33).

Subsequently or simultaneously, the carbanion portion of the organometallic ion-pair attacks an adjacent hydrogen atom, removing it as a proton. The coordination of the metal cation with the hetero element weakens the ionic bond between the metal and the carbanion and at the same time polarizes the adjacent carbon-hydrogen bond as to facilitate proton removal.

Mechanism 2

The other interpretation of the reaction stresses the electrophilic properties of the organometallic molecule and pictures the initial coordination with a hetero atom as being followed, or accompanied, by an electrophilic attack of the metal cation on the carbon atom from which hydrogen is to be removed⁶¹. The role of the carbanion in removing the proton is pictured as being of secondary importance. This theory is in accord with the view that organoalkali compounds generally function as electrophilic species⁶².

In 1979, Gschwend and Rodriguez⁶³ reported that "*...Although numerous studies on the mechanism of heteroatom-facilitated lithiations have been undertaken, particularly those of alkyl aryl ethers, most of the current views are based more on speculation and hypothesis than on data...*". An understanding⁶³ of the mechanism of metalation can be facilitated by the recognition of the existence of two distinct types of metalating agents. The first type, lithium alkyls and lithium aryls, are oligomers of varying complexity in solution. In addition they are electron-deficient species, and therefore Lewis acids which can coordinate with Lewis bases such as ethers and amines with consequent depolymerization to varying extents. It is more important to note that kinetically these reagents become more basic as the aggregate size diminishes; therefore, tetrahydrofuran is the solvent of choice for generating reactive species.

The distinguishing characteristic of the second type of lithiating reagent is its negligible extent of Lewis-acid character relative to uncomplexed lithium alkyls and lithium aryls, eg. *n*-butyllithium / amine complexes and lithium dialkylamides. Also the lithium

dialkylamide complexes possess decreased thermodynamic basicity relative to lithium alkyls.

The phenomenon of an increased kinetic basicity of heteroatom bases in general and lithium amides in particular, may be rationalized by the availabilities of a free pair of

Figure 34

electrons, which permits the formation of a four-membered transition state (fig 34). The intermediacy of a free carbanion is thus avoided.

Most mechanistic studies of the *ortho*-lithiation reaction have been carried out with alkyl aryl ethers, where a combination of the two mechanistic determinants, *i.e.* coordinative and inductive effects, is operative. NMR data have shown that lithiation in hydrocarbon solvents results in the initial disruption of the oligomeric alkyllithium to yield a reactive complex with the substrate. The stoichiometry of the complex depends on the structure of the ether. In the intermediate, in the process (fig 35), the carbon - lithium

Figure 35

bond of the metalating agent and the carbon - hydrogen bond of the substrate are polarized to a greater extent, thus rendering the proton to be removed more acidic by induction. The second step, namely the actual deprotonation (protophilic attack), is rate determining.

In a more sterically hindered case⁵³, the metalation of phenyl *t*-butyl ether **148** with *t*-butyl lithium, the major product was *ortho*-metalation (fig. 36) with less than 1% at any

Figure 36

other site. With *n*-butyl lithium there was no *meta*-metalation observed.

Parham and Anderson⁶¹ reported that the use of the ether as a protecting group has the disadvantage that the product obtained after the metalation is still an ether and hence the conditions for its cleavage are often so vigorous that considerable loss by decomposition may result during the deprotection. The authors utilized a THP ether as a protecting group and it also served as an *ortho*-directing agent in metalation (fig. 37). The authors reported that in most cases metalation, and subsequent carbonylation, followed by hydrol-

Figure 37

ysis gave the corresponding hydroxy acid in 48% to 60% yield.

A similarly protected catechol should be capable of being metalated in the 3-position.

Synthesis of 3-chlorocatechol

The two di-THP ether diastereomers **151** of catechol were prepared, in 49% yield, by reacting catechol with 4.5 eq. of dihydropyran (DHP) and a catalytic amount of *p*-tolue-

Figure 38

nesulphonic acid (fig. 38).

The metalation of the di-THP ether was performed with *n*-BuLi and various chlorinating reagents (fig. 39). The results of the reactions are shown in Table 16.

Figure 39

Table 16

Results obtained in the Chlorination of di-THP ether of Catechol

<u>Metalating Conditions</u>	<u>Temperature</u>	<u>Chlorinating Reagent</u>	<u>Product(yield)</u>
<i>n</i> -BuLi / TMEDA (3 eq)	0°C	CCl ₃ SO ₂ Cl (3.2 eq)	tars
<i>n</i> -BuLi / TMEDA (1.1 eq)	-98°C	CCl ₃ SO ₂ Cl (1.1 eq)	tars
<i>n</i> -BuLi / TMEDA (1.1 eq)	-116°C	CCl ₃ SO ₂ Cl (2 eq)	tars
<i>n</i> -BuLi / TMEDA (3 eq)	-140°C	CCl ₃ SO ₂ Cl (3.2)	tars
<i>n</i> -BuLi (3.6 eq)	25°C (1.5 hr)	NCS (2 eq)	3-chlorocatechol di-THP(10)
<i>n</i> -BuLi (3.6 eq)	25°C (2.5 hr)	NCS (4.4 eq)	3-chlorocatechol di-THP(71)

The poor yields of the chlorination using trichloromethanesulphonyl Chloride can be attributed to the reactivity of the trichloromethanesulphonyl Chloride. N-chlorosuccinimide (NCS) is a milder chlorination reagent. The identity of the product was confirmed by MS and NMR. All attempts to hydrolyse the THP ether by standard methods (oxalic acid in methanol, or 1% HCl in THF ⁶⁴) failed and produced only tars. It is possible that the THP ethers were cleaved, but the catechols were oxidized during the course of the reaction.

Other methods which were attempted but, in this study, did not produce the desired materials are shown in scheme 6.

Scheme 6

Scheme 6a

Figure 40

Synthesis of Dioxins by the Catechol Method

Dioxins may be produced by reacting chlorinated catechols with chlorobenzenes or chloronitrobenzenes. The only requirement on the reaction is that the chlorobenzene or chloronitrobenzene must possess an *ortho*-chloro group (fig. 41).

Figure 41

The problems with the polychlorobenzene reaction are the long reaction times (some reactions required one week) and the high reaction temperatures. These factors combine to yield products which contain lower chlorinated species due to the loss of chlorines during the course of the reaction.

The major impurity found, in products produced by the polychloronitrobenzene reaction, is the starting polychloronitrobenzene. This impurity may be easily removed by ptlc or digesting the product in hot benzene. These reactions were typically faster than the corresponding polychlorobenzene reaction; most reactions were complete in two to five hours at reflux.

Table 17 indicates the dioxins which have been produced using both of these methods.

Table 17

Dioxins synthesized by the catechol method

Catechol	polychloro nitrobenzene*	Dioxin	conditions**	chemical yield	purity	ref.
4-chloro	2,4,5-triCNB	2,3,7	A	X	X	19
4,5-dichloro	2,5-diCNB	2,3,7	A	X	X	19
4-chloro	2,4,5-triCNB	2,3,7	B or C	17%	99%	19
catechol	hexaCB	1,2,3,4	-	55%	-	65
			-	55%	97.1%	66
3-chloro	2,3,4,5-TCNB	1,2,3,6/9	-	41%	99%	66
3,4,5-trichloro	2,3-diCNB	1,2,3,6/9	-	5%	-	65
4-chloro	2,3,4,5-tetraCNB	1,2,3,7/8	-	20%	-	65
			-	20%	97.3%	66
3-chloro	2,3,5,6-tetraCNB	1,2,4,6/9	-	42%	-	65
			-	42%	97.9%	66
4-chloro	2,3,5,6-tetraCNB	1,2,4,7/8	-	25%	-	65
			-	25%	95.5%	66
3,4-dichloro	2,3,4-triCNB	1,2,6,7/8,9	-	20%	-	65
			-	20%	100%	66
3,5-dichloro	2,3,4-triCNB	1,2,6,8/7,9	-	33%	-	66
			-	33%	-	66
3,5-dichloro	2,3,6-triCNB	1,2,6,8/7/9	-	12%	-	65
		and 1,3,6,9	-	12%	100	66
3,6-dichloro	2,3,6-triCNB	1,2,6,7/1,4,6,9	-	5%	-	65
			-	5%	-	66
4,5-dichloro	2,3,6-triCNB	1,2,7,8/1,4,7,8	-	41%	-	65
3,5-dichloro	2,3,5-triCNB	1,3,6,8/7,9	-	37%	98%	66
3,5-dichloro	2,4,5-triCNB	1,3,7,8	-	37%	-	65
			-	41%	97.6%	66
4,5-dichloro	1,2,4,5-tetraCB	2,3,7,8	-	8%	-	65
			-	8%	93.7%	66
4,5-dichloro	2,3,4,5-tetraCNB	1,2,3,7,8	A	29%	98%	19
4,5-dichloro	2,3,5,6-tetraCNB	1,2,4,7,8	A	18.5%	99%	19
3,4,5-trichloro	3,5,6-triCNB	1,2,3,6,7,9/6,8,9	D	27%	88%	19
4,5-dichloro	pentaCNB	1,2,3,4,7,8	A	44%	98%	19
3,4,5,6-tetrachloro	3,4,5-triCNB	1,2,3,4,6,7,9	A	5%	93%	19

* CB = chlorobenzene CNB = chloronitrobenzene

** A = K_2CO_3 / Acetone B = K_2CO_3 / DMSO
C = K_2CO_3 / HMPA D = K_2CO_3 / 18-crown-6 / Acetonitrile

- indicates that no data was reported

Dioxins synthesized in this study *via* the condensation of polychlorocatechols and polychlorobenzenes

The reaction of 4-chlorocatechol **127** and 2,3,4,5-tetrachloronitrobenzene **118**, was studied in an attempt to determine the optimum conditions for the synthesis of 1,2,3,7/8-TCDD **32** / **33** (fig. 42).

The reaction of **127** and **118** in which the catechol salt was formed *in situ* by adding of K_2CO_3 to the acetone solution, gave after six hours at reflux, a mixture of tri- and tet-

Figure 42

raCDD (4 : 1) with the major impurity being the starting chloronitrobenzene 118. The chloronitrobenzene was removed by digesting the precipitate in hot benzene. The GC / MS indicated not one tetraCDD but two tetraCDDs in a ratio of 5 : 1, while the ratio reported by Gelbaum *et al*⁶⁶ was about 2 : 1 (1,2,3,7 : 1,2,3,8).

The reaction was repeated with the addition of a catalytic amount of 18-crown-6. The GC / MS, after work-up, indicated that the sample was very pure and the only impurity found was a phthalate ester which probably came from the cap of the sealed vial.

In an effort to determine if the product could be purified more efficiently by sublimation, instead of digesting the crude precipitate, the crude precipitate was sublimed at 180°C to 250°C. To eliminate the acids and chromium (from chromic acid bath) which might react with the compound during the sublimation, the apparatus was soaked in an 1% EDTA solution. The apparatus was then rinsed 10 times and soaked overnight in de-ionized water, before drying. The GC / MS of the sublimed dioxin gave only one peak which indicated that sublimation could be used to produce very pure dioxin but in low yields.

The synthesis of 1,2,3,4,7,8-hexaCDD 69 was accomplished in a 52% yield (fig. 43) and this increase in the yield over the reported method (the condensation of 4,5-dichlorocatechol with pentachloronitrobenzene) could have resulted from the purification method which was used.

Figure 43

The reaction was worked-up by adding a 50% MeOH / H₂O solution to the reaction mixture. The precipitate was then collected and then digested directly in hot benzene. The solid was then collected and the GC / MS of this material indicated 5 peaks (a phthalate bleed peak from the column was also noted - Table 18). The GC/MS indicated the purity of the HexaCDD to be greater than 99%.

Table 18

1,2,3,4,7,8-HexaCDD synthesis

Retention time arbitrary units	GC Intensity	% Intensity	Identity of peak
305	1069	0.59	pentachloronitrobenzene
531	347	0.19	unknown
608	179440	99.2	1,2,3,4,7,8-hexaCDD
627	ND	ND	pentachloronitroDD
630	ND	ND	hexaCDD isomer
708	ND	ND	unknown

ND - peak did not exceed the threshold level for the detection system

The synthesis of 1,2,3,7,8-pentaCDD 57 was accomplished in 75% yield, by the condensation of 4,5-dichlorocatechol 129 and 2,3,4,5-tetrachloronitrobenzene 118 (fig. 44).

Figure 44

The product possessed a minor impurity which was a second pentachlorodibenzo-*p*-dioxin which probably resulted from a small amount of a second tetrachloronitrobenzene. The purity of the product was 95% by GC / MS.

Table 19 indicates the dioxins which were produced by this method and from this table the optimum conditions for all the dioxins are the same except for the solvent. For the higher dioxins (> 3 chlorines in the dioxin) acetone was the best solvent and DMSO for the lower dioxins (3 or less chlorines in the dioxin).

Table 19

Entry	Catechol	Dioxins produced in this study			
		polychloro nitrobenzene	dioxin	%chemical yield (lit)	%purity (lit)
A	0	1,2-di*	unsub	23(NR)	-(NR)
A	0	2,6-di	1	20(NR)	92.3(NR)
A	0	2,4-di	2	11(NR)	76(NR)
A	0	2,4,5-tri	2,3	70(81)	98(NR)
A	4-chloro	2,4,5-tri	2,3,7	21(17)	99(99)
B	4-chloro	2,3,4,5-tetra	1,2,3,7/8	24.8(20)	>99(97.3)
B	4,5-dichloro	2,4,6-tri	1,3,7,8	42(NR)	98(100)
B	4-chloro	penta	1,2,3,4,7	47(NR)	98(NR)
B	4,5-dichloro	2,3,4,5-tetra	1,2,3,7,8	75(29)	95(98)
B	4,5-dichloro	2,3,5,6-tetra	1,2,4,7,8	60(18.5)	99(99)
B	4,5-dichloro	penta	1,2,3,4,7,8	52(44)	99.2(98)

* in this experiment 1,2-dichlorobenzene was used

Entry - A - reaction performed in DMSO

- B - reaction performed in Acetone

- NR - not reported in the literature reference

The purity of the products were improved by digesting the product in benzene after filtering the reaction mixture but the yields were slightly less than those reported in the literature. The use of sublimation to purify the reaction product, did increase the purity of the dioxin but the yield was very low.

Nucleophilic Displacement of Aromatic Nitro Groups

The displacement of a nitro group has been reported by our laboratory as a method of synthesizing dioxins (fig 45) ⁶⁷, and the synthetic use of nitro displacement has been reviewed in the past ⁶⁸.

Figure 45

Many groups have performed kinetic studies to determine the relative rate of displacement of an activated aromatic nitro group in comparison with other functionalities (such as chlorine, bromine, fluorine and similar functionalities, Table 20)

Table 20

**Table of the relative rate of displacement of the
nitro group with various nucleophiles**

	<u>nucleophile</u>	<u>Rate of exchange</u>	<u>Ref.</u>
piperidine in MeOH	$\text{NO}_2 \approx 200 \times \text{Cl}$ $\approx 200 \times \text{Br}$ $\approx 0.25 \times \text{F}$	69
	MeO^- in MeOH	$\text{NO}_2 \approx 400 \times \text{Cl}$	71
MeO^- in MeOH	$\text{Me}_2\text{S}^+ > \text{Me}_3\text{N}^+ > \text{F} \approx \text{NO}_2 > \text{Cl}$	70
	piperidine in DMSO	$\text{NO}_2 \approx 0.02 \times \text{F}$ $\approx 9 \times \text{Cl}$	72
EtOH and	$\text{NO}_2 \gg \text{F} > \text{Cl}$	71

The synthetic utility of nucleophilic displacement of a nitro group activated by an *ortho* or *para* function, other than nitro, was demonstrated by Tiemann ⁷³, in the synthesis of 2-chloro-4-methoxybenzaldehyde, **155**, from 2-chloro-4-nitrobenzaldehyde, **154** (fig. 46), and by Ringer *et al* ⁷⁴ in the synthesis of *o*- and *p*-methoxybenzonitrile, **157** and **159**, from *o*- and *p*-nitrobenzonitrile, **156** and **158** (fig. 47).

Figure 46

Figure 47

Gorvin *et al* ⁷⁵ reported that, in dimethyl sulfoxide (DMSO), substituted benzophenones **160**, **161**, and **162** reacted with stoichiometric amounts of methanolic sodium methoxide

to yield **163**, **164** and **165** via isolable intermediates such as **166** and **167** (fig. 48).

Figure 48

In the case of 2,2',4,4'-tetranitrobenzophenone, **162**, the *p*-nitrogroup were preferentially displaced, so that **168** was isolated in yields of > 80%. Subsequently, a third nitro group was displaced to give **165** but the remaining nitro group was not displaced (fig. 49).

Figure 49

The authors ⁷⁵ also reported on the displacement of nitro groups in the benzophenones by primary aromatic amines. They found that the 4,4'-dinitrobenzophenone, **160**, was unaffected by aniline, even under refluxing conditions, whereas the 2,2'-dinitrobenzophenone **161** reacted with refluxing aniline with displacement of one nitro-group and with the slow replacement of the second nitro group. The tetranitrobenzophenone **162** reacted rapidly with aniline, displacing both ortho nitro groups to yield 3,6-dinitro-10-phenyl-9-acridone **169** in yields of about 80% (fig. 50).

Figure 50

Gore *et al* ⁷⁶ reported on the displacement of a nitro-group in 1,3,5-trichloro-2,4,6-trinitrobenzene **170**, with lithium chloride in acetone or *iso*-propyl alcohol, to yield 1,2,3,5 tetrachloro-4,6-dinitrobenzene **172** (fig. 51).

The authors rationalized the reaction by stating that in the trinitrobenzene **170**, the three equivalent nitro-groups cannot be co-planar with the aromatic ring. The σ -complex transition state **171** for the reaction would permit relief of steric crowding at C₂. Which would result in more effective charge-delocalisation involving the nitro-groups at C₄ and C₆.

Figure 51

Use of nitro displacement in Dioxin synthesis

In this study, it was found that by the use of a suitable polychlorodinitrobenzene, it is possible to produce PCDDs in chemical yields of at least 80% and with chemical purities of greater than 85%, directly from the reaction mixture (Table 21).

Table 21

Synthesis of dioxins by the reaction of Chlorocatechols with
2,5-dichloro-1,4-dinitrobenzene **174**

Catechol	%Chemical Yield based on GC/MS Nitrodioxin chlorodioxin		%Overall Chemical Yield
catechol	10	85	80
4-chlorocatechol	9	90	82
4,5-dichlorocatechol	2	97	95

The major impurity found was the starting polychlorodinitrobenzene, which was easily removed by digesting the crude precipitate in hot benzene. The final purity of the material obtained was greater than 95% (by GC/MS).

This reaction is interesting because after the formation of the nitrodioxin **175**, the nitro-group has been displaced by the chloride ion which is present after the initial coupling of the catechol and the nitrobenzene. Also, interesting is the rate of displacement decrease as the number of chlorine groups decreases on the dioxin A ring. The reaction can be rationalized by following the intermediates proposed in the above reactions.

The reaction of 2,5-dichloro-1,4-dinitrobenzene **174** with chlorinated catechols **173** was found to produce fully chlorinated dioxins **176** as well as a small amounts (<10%) of nitrodioxins **175** (fig 52).

When the nitrodioxin is attacked by the chloride ion, a possible intermediate formed is **175A** (fig. 53), which is similar to that of Gore ⁷⁶.

The increase in the yield of chlorodioxins **176** can be rationalized by noting that the reaction yield will be increased by stabilization of the intermediate ⁷⁷.

The addition of chlorine to the A ring, increases the dipole which can remove electron density from the aromatic ring, which will in turn remove electron density from the oxygens, and in turn will allow electron density to be removed from the ring undergoing substitution (fig. 54).

Electron-density is removed
by the chlorines and this
stabilizes the intermediate

Figure 54

Some dipole calculations for the three nitrodioxins are shown in Table 22. In the case of 2,3,7,8-TCDD, the two additional chlorines on the A ring have removed electron density which will stabilize the intermediate and the steric strain in the molecule will be reduced if the nitro group is replaced by the chlorine.

Table 22

Calculated Dipole Moments for the Nitrodioxin Intermediates using TRIBL⁹²

#of Chlorines (<u>position on A ring</u>)	Dipole (D)	X	Dipole Y	Z
0	3.967	3.9043	0.7019	0
1(7)	2.521	2.5051	0.2388	0
2(7,8)	1.468	1.2368	0.7901	0

To determine the scope of this reaction a number of dinitro polychlorobenzenes were reacted in a similar fashion with catechols (fig 55).

In the reaction of 1,4-dinitro-2,3,5,6-tetrachlorobenzene **177** with 4-chlorocatechol **127**, the product isolated was 3-nitro-1,2,4,7-tetrachloroDD **178**, in a 59% yield and 92% pure. The major impurity was the starting material, 1,4-dinitro-2,3,5,6-tetrachlorobenzene **177**.

In the reaction of tetrachlorocatechol **134** with 1,4-dinitro-2,3,5,6-tetrachlorobenzene **177**, only the 1,3,4,6,7,8,9-heptachloro-2-nitrodioxin **179** was isolated in a 20% yield.

By the use of TRIBL⁹² we can determine the charges present at each carbon.

TRIBL - is a molecular modeling program, QCPE 529

Figure 55

Table 23

AM1 calculations to determine Charge at the Carbon with the Nitro group attached

<u>Dioxin</u>	<u>Charge at Nitro Carbon</u>	<u>% Conversion to chlorinated dioxin</u>
2-nitro-3-chloro	-0.1311	85
2-nitro-3,7-dichloro	-0.1285	90
2-nitro-3,8-dichloro	-0.1306	90
2-nitro-3,7,8-trichloro	-0.1291	97
2,4,6-trichloro-1,3,5-trinitro	-0.1020	§
	-0.1047	
	-0.1032	
2-nitro-1,3,4,8-tetrachloro	-0.0596	ND
2-nitro-1,3,4,7-tetrachloro	-0.0602	ND
2-nitro-1,3,4,6,7,8,9-heptachloro	-0.0598	ND

§ the amount of the nitro compound converted was not reported however conversion did occur with a reported rate of $K_2 = 0.525 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ at 298°K.

The data in Table 23 indicates that the reactions which can undergo substitution may be predicted in advance. With this information, one can predict which nitro groups can be utilized synthetically to undergo substitution by chloride and possibly other ions.

Summary

There are two methods to produce dioxins, the pyrolysis of phenols or the reaction of catechols with polychlorinated benzenes. The synthesis of dioxins *via* the pyrolysis of phenolates suffers from the Smiles rearrangement, low yields and the possibility of multiple products (see Table 10), if more than one chlorine is *ortho* to the phenol. The multiple products which are produced by this method include lower chlorinated dioxins (compounds where one or more chlorines have been lost) which are difficult to separate by most physical methods.

The problems of the phenolate method make it synthetically useful only for those dioxins which are symmetrical and only one dioxin can be produced.

However, the method of Grey *et al* ¹⁹, the reaction of a suitably substituted catechol and a polychlorobenzene (or polychloronitrobenzene) gave dioxins in higher chemical yields (~15 - 55%) than the phenolate method and the major impurity in the catechol method is the starting polychlorobenzene. The purity of most of the dioxins was greater than 95% and a number of them were produced at greater than 98%, with minimal purification. The major impurity, which was determined by GC/MS, was the starting polychlorobenzene.

Many groups have shown that aromatic nitro groups may be displaced ^{69,76} by various nucleophiles and our laboratory has utilized this reaction in the synthesis of dioxins (see Table 22). These dioxins were produced in high yields, 80 - 95% yields, with little purification required. It was found that highly chlorinated dioxins could not be produced by this method. The reaction stopped at the polychlorinated nitrodioxin, but the dioxins which did not undergo displacement at the nitro group possessed similar electrondensities at the carbon where the displacement occurs. There is an indication, by the use of semi-empirical molecular orbital calculations, that the removal of electron density is a requirement for the stabilization of the intermediate form ¹ during the displacement of the nitro-group. A correlation was noted between the nitro groups which were displaced and the

electron density at the carbon attached to the nitro group. The nitro groups which were displaced, were attached to carbons which possessed a charge of -0.1032 to -0.1311. The nitro groups which were not displaced possessed a charge of -0.0596 to -0.0602. This indicates that it may be possible to predict which materials will undergo the displacement.

The release of steric strain in the molecule, by the displacement of the bulky nitro group, should favour displacement in the highly chlorinated dioxins. Further study of this reaction could be performed on smaller model compounds.

Chapter 2

Development of an Improved Radioimmunoassay (RIA) for Dioxins

Development of a Radioimmunoassay for Dioxin⁷⁹

The reason for the development of a radioimmunoassay (RIA) for the detection of polychlorinated dibenzo-*p*-dioxins (PCDDs) is to, by means of a pre-screening test, eliminate PCDD free samples from further conventional (and more costly) analysis.

Conventional methods for the determination of PCDDs in environmental samples are time consuming and expensive, a single dioxin analysis with isomer confirmation, can cost in excess of \$2000.00.

An environmental accident, involving the release of PCDDs into the environment, could create a demand for the rapid analysis of many samples, which could not be handled by the present facilities, only samples which indicate the presence of dioxins need to be submitted for single dioxin analysis.

Radioimmunoassay techniques are widely used in chemical laboratories for the detection and determination of antibodies, hormones and ~~medications~~; as exemplified below :

Radioimmunoassays currently in use⁸⁰

<u>Steroids and Drugs</u>	Aldosterones	<u>Pesticides</u>	Dieldrin
	Corticosterones		Aldrin
	Cortisol		2,4-dichlorophenoxyacetic acid
	5 α -dihydrotestosterone		2,4,5-trichlorophenoxyacetic acid
	17 β -Estradiol		
	17 α -Hydroxyprogesterone		
	Progesterone		
	Testosterone		
	Prostaglandin		
	Tetrahydrocannabinol		

The method of radioimmunoassay has been extensively described by T. Chard and will be discussed in the following section.

Radioimmunoassay ⁷⁸

The work of Yalow (who shared the 1977 nobel prize in recognition of her role in the development of the radioimmunoassay) and Berson in 1960 on radioimmunoassay (RIA) represented a major advance in biological measurement. The major development in this area was the extension of the RIA into the analysis of small molecules which are not immunogenic themselves. But when the molecules are conjugated to a protein, antibodies may be generated to these conjugated materials.

The technique of radioimmunoassay can attribute its wide acceptance to four factors:

- 1) the assay is generally, more sensitive, more specific and easier to perform than by classical methods
- 2) it can be applied to substances for which there exists no other means of analyzing eg. hormones, drugs, proteins etc.
- 3) the same system can easily be adapted, by a skilled technician, to other compounds
- 4) many radioactive isotopes possess energies intense enough to allow the detection of a few atoms with relatively simple equipment

The basic mechanism ⁷⁸ of a radioimmunoassay is shown in fig 56 which shows binding of antigen to antibody in the presence of different total masses of antigen. The distribution

If equal amounts of antibody and antigen are allowed to react together (i) then at equilibrium they will form an antigen-antibody complex (area B) together with a proportion of both the antigen (area F) and antibody which remains free. If the amount of antibody is held constant while the total amount of antigen is increased (ii) then, at equilibrium the amount of antigen-antibody complex (B) is increased, however, the increase in the free fraction (F) is relatively larger and thus yields a lower bound to free ratio.

Figure 56⁷⁸

3

OF/DE

3

MicroD

N-Methyl 4-methoxyphenethylamine⁹⁴ 226

Benzaldehyde (10.5 g, 99 mmol) was added to 4-methoxyphenethylamine (15 g, 99 mmol) and a reaction occurred immediately. The resulting water was removed under reduced pressure, then benzene (10 mL) was added to azeotrope off any remaining water. Freshly distilled dimethyl sulfate (12.7 g, 101 mmol) in benzene (25 mL) was added to the reaction mixture and the reaction was heated to 90°C on a water bath for thirty minutes. Two phases were not noted (too much water was removed, therefore added 7 mL of water) and allowed the reaction to stir for twenty minutes. The solution was separated and the aqueous phase was extracted with benzene (30 mL), dissolved in 80% ethanol (150 mL). Then the ethanol solution was refluxed for thirty minutes. After thirty minutes, water (10 mL) was added to the reaction mixture and the ethanol was removed under reduced pressure. The aqueous solution was made acidic with concentrated hydrochloric acid, whereupon the solution turned green. The aqueous solution was extracted with ether (2 X 30 mL) and the aqueous solution was made basic with 4N potassium hydroxide. The aqueous solution was extracted with ether (3 X 50 mL) and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the resulting oil was distilled (123°C / 12 mmHg (lit. 127°C / 9 mmHg)) to yield the desired amine (13.2 g, 80 mmol, 81%).

¹H NMR (DMSO d-6) δ = 2.3 (N-CH₃, S, 3H)

δ = 2.7 (CH₂, S, 2H)

δ = 2.9 (CH₂, S, 2H)

δ = 3.5 (O-CH₃, S, 3H)

δ = 7.0 (Ar-H, q, 4H)

^{13}C NMR (CDCl_3)

IR (film) : 3375 cm^{-1} (NH), 1610 cm^{-1} (C=C)

MS (m/e, CI/ether) : 166 ($\text{M}^+ + 1$)

N-Methyl 4-(*p*-methoxyphenethylcarbamoyl) butanoic acid **227**

To a stirred solution of N-methyl 4-methoxyphenethyl amine **226** (130.5 mg, 0.79 mmol) in benzene (5 mL), was added imidazole (65.0 mg, 0.95 mmol) followed by glutaric anhydride (0.123 g, 1.1 mmol), and the reaction was stirred overnight at room temperature.

After twenty-three hours, 1M HCl (30 mL) was added and the reaction was stirred for 5 minutes. The reaction mixture was extracted with chloroform (3 X 30 mL) and the organic layer was washed with brine (2 X 25 mL) and was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the resulting oil was purified by ptlc (20 X 20 cm, 1mm, 15% methanol / chloroform) to yield five bands. The product was obtained as a colourless oil ($R_f = 0.6$, 198.0 mg, 0.71 mmol, 90%).

^1H NMR (DMSO d_6) $\delta = 2.87$ and 2.94 (N-CH₃, S, doubling due to amide, 3H)

$\delta = 1.8$ - 2.8 (CH₂, m, 6H)

$\delta = 3.4$ - 3.8 (CH₂, m, 4H)

$\delta = 3.7$ (O-CH₃, S, 3H)

$\delta = 7.0$ (Ar-H, q, 4H)

$\delta = 10.7$ (O-H, brS, 1H)

^{13}C NMR (benzene- d_6)

IR (film) : 3500 - 2500 cm^{-1} (COOH), 1725 cm^{-1} (acid), 1610 cm^{-1} (amide)

MS (m/e, CI/ether) : 280 ($\text{M}^+ + 1$)

N-p-Methoxyphenethyl *N*-methyl *N'*-phenyl glutaramide **229**

Using thionyl chloride

To a stirred solution of the acid **227** (100 mg, 0.36 mmol) in benzene (5 ml) was added thionyl chloride (0.3 ml) and the solution was stirred at room temperature overnight. After twenty hours, pyridine (0.3 mL) and aniline (0.3 mL, 306 mg, 3.2 mmol) were added and the reaction stirred for two hours. After two hours, 5% HCl (30 mL) was added and the reaction was stirred for 5 minutes. The reaction was extracted with chloroform (3 X 30 mL) and the organic layer was washed with brine (2 X 25 mL) and was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the resulting oil was purified on ptlc (20 X 20 cm, 2 mm, 15% methanol / dichloromethane) to yield an intensely red coloured oil. The oil was purified on an alumina plate (20 X 20 cm, 1mm, dichloromethane) to give two bands ($R_f = 0.02$ and $R_f = 0.4$) of which, the upper band was the desired material (21.5 mg, 0.06 mmol, 17%).

Using Oxalyl Chloride

To a stirred solution of the acid **227** (100 mg, 0.358 mmol) in acetone (5 ml) was added oxalyl chloride (0.3 ml) and the solution was stirred at room temperature for thirty minutes. After thirty minutes, pyridine (0.3 mL) and aniline (0.3 mL, 306 mg, 3.24 mmol) were added and the reaction stirred for two hours. After two hours, 5% HCl (30 mL) was added and the reaction was stirred for 5 minutes. The reaction was extracted with chloroform (3 X 30 mL) and the organic layer was washed with brine (2 X 25 mL) and was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the resulting oil was purified on ptlc (20 X 20 cm, 2 mm, 15% methanol / dichloromethane) to yield an oil (16.1 mg, 0.046 mmol, 12%).

Using ethylchloroformate

To a stirred solution of the acid **227** (100 mg, 0.36 mmol) in benzene (5 ml) was added ethylchloroformate (0.3 ml) and the solution was stirred at room temperature for thirty minutes. After thirty minutes, pyridine (0.3 mL) and aniline (0.3 mL, 306 mg, 3.24 mmol) were added and the reaction stirred for two hours. After two hours, 5% HCl (30 mL) was added and the reaction was stirred for 5 minutes. The reaction was extracted with chloroform (3 X 30 mL) and the organic layer was washed with brine (2 X 25 mL) and was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the resulting oil was purified on ptlc (20 X 20 cm, 2 mm, 15% methanol / dichloromethane) to yield an oil (64.4 mg, 0.18 mmol, 48%).

MS (m/e, CI/ether) : 355 ($M^+ + 1$)

N-3,7,8-Trichlorodibenzo-*p*-dioxin N'-*p*-methoxyphenethyl N'-methyl glutaramide 222

To a stirred solution of the acid **227** (29.6 mg, 0.11 mmol) in benzene (5 ml) was added ethylchloroformate (1 ml of 0.101 mL / 7 mL solution in benzene) and the solution was stirred at room temperature for thirty minutes. After thirty minutes, an aliquot (0.7 mL) of the carbonate solution was diluted with benzene (5 mL) and pyridine (0.18 mL) and 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin **182** (4.0 mg, 12.1 μ mol) were added and the reaction stirred for two hours. After two hours, a second aliquot (0.7 ml) of the carbonate was added to the solution and the reaction was stirred for six hours. After six hours, 5% HCl (30 mL) was added and the reaction was stirred for 5 minutes. The reaction was extracted with chloroform (3 X 30 mL) and the organic layer was washed with brine (2 X 25 mL) and was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the resulting oil was purified on ptlc (20 X 20 cm, 2 mm, 15% methanol / dichloromethane) to yield an amorphous solid (1.5 mg, 2.7 μ mol, 22%).

Repeat

A solution of the acid **227** (136 mg, 0.49 mmol) in ethanolic potassium hydroxide (0.756 mL of a solution of 40 mg / mL) and ethanol (4 mL) was reacted at room temperature for five minutes then the solvent was removed under reduced pressure. Then acetone (5 mL) was added to the solid precipitate and was removed under reduced pressure. The solid was cooled in an ice bath and the solid was suspended in ether (25 mL). the reaction was stirred for five minutes the oxalyl chloride (1.08 mL) was added and the solution was stirred at 0°C for two hours. After two hours, the solution was filtered and the solvent was removed under reduced pressure. The precipitate was resuspended in carbon tetrachloride (15 mL) and pyridine (1.0 mL) and 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin **182** (4.0 mg, 12 μ mol) were added and the reaction stirred overnight. After twenty hours, 5% HCl (30 mL) was added and the reaction was stirred for 5 minutes. The reaction was extracted with chloroform (3 X 5 mL) and the organic layer was washed with brine (2 X

5 mL) and was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the resulting oil was purified on ptlc (20 X 20 cm, 0.25 mm, 15% methanol / dichloromethane) to yield an amorphous solid (5.8 mg, 11 μ mol, 85%).

MS (m/e) : molecular ion not detected

301.1 (amino dioxin fragment)

285.1 (amino dioxin fragment)

266.1 (amino dioxin fragment)

239.1 (amino dioxin fragment)

149.1 (N-methyltyramine fragment)

134.2 (N-methyltyramine fragment)

121.2 (N-methyltyramine fragment)

4-Hydroxy-3-iodophenethylphenylamine

The 4-acetoxyphenethylphenylamine (10 mg, 0.03 mmol) was dissolved in dimethylformamide (3 mL) and sodium iodide (6.61 mg, 0.04 mmol) was added, with stirring. Chloramine-T (12.4 mg, 48 μ mol) was added to the solution and the solution turned yellow instantly.

After two hours, the reaction was diluted with water (5 mL) was acidified with 5% HCl (5 mL), the reaction turned dark brown and sat'd sodium metabisulfite (3 mL) was added. The solution turned light yellow and the reaction was extracted with ethyl acetate (10 mL). The organic layer was washed with brine (2 X 5 mL) and the organic layer was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the residue was purified by ptlc (20 X 20 cm, 0.25 mm, 50% ethyl acetate / hexane developed two times) (7.3 mg, 28 μ mol, 90%).

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Appendix A

The relationship between $\text{Log } K_{(\text{octanol} / \text{water})}$ and the environmental fate of organic compounds

The relationship between Log K_{OW} and various physical properties ⁹⁶

The K_{OW} has been recognized as a key parameter in modelling the environmental fate of organic compounds. The theory behind the relationship of K_{OW} and water solubility, organic matter adsorption, soil adsorption and Bioconcentration Factors has been shown by Briggs ⁹⁶ and is described below.

Briggs ⁹⁶ noted that the "hole" theory of solubility considers that, in order to dissolve, a solute must make a hole in the solvent. The parachlor (P) is one measure of molar volume and thus of the size of the hole. The bigger the molecule, the larger the hole and the lower the solubility unless there are H-bonding or other solute - solvent interactions.

The driving force for partition between two immiscible solvents is the difference in the energy required to make a hole in the two phases. Neglecting any interactions, Lambert ⁹⁷ derived an equation relating the parachlor to the partition coefficient,

$$\log K = aP \Delta U \quad (1)$$

$$a = \text{constant}$$

$$\Delta U = \text{difference in internal pressure between the two phases}$$

Where water is one of the phases, the ΔU term is dominated by the internal pressure of water which is ~10 times that of immiscible organic solvents. Except where the organic phase contains much water (>5M water), since if the presence of water in the organic phase increases the internal pressure in the organic phase and hence decrease the difference between the two phases and decrease K), then equation (1) becomes, if we assume that the difference in internal pressure ΔU is proportional to the difference in the water concentrations in the aqueous and organic phase, ΔW ,

$$\log K = a' P \Delta W. \quad (2)$$

Since hydrogen bonding is generally the most important solute - solvent and if we assume that H - bonding in a phase is proportional to the water concentration, W , in that phase then the contribution of hydrogen bonding to the solvent - water partition coefficient will be W_1 / W_{aq} for organic solvent 1 and equation (2) becomes

$$\text{Log } K_1 = a' P \Delta W_1 + \log(W_1 / W_{aq}). \quad (3)$$

So that the Collander relationship between K_1 for solvent 1 and K_2 for solvent 2 can be obtained, $a' P$ can be eliminated to give,

$$\log K_1 = [\log K_2 - \log(W_2 / W_{aq})] \Delta W_1 / \Delta W_2 + \log(W_1 / W_{aq}) \quad (4)$$

For organic liquids, water solubility can be considered as a partition of the chemical between itself and water. The partition coefficient describing this is,

$$\log K = \log(C_L / WS) = \log C_L - \log WS \quad (5)$$

where C_L is the concentration of the liquid itself and WS is the molar water solubility. For most low molecular weight compounds C_L is in the range 5 - 10 so that $\log C_L$ is ~ 1 and equation 5 becomes,

$$\log K = 1 - \log WS. \quad (6)$$

By making the simplifying assumption the H-bonding interactions in the liquid and octanol are comparable gives from equation 4,

$$\log K = 1 - \log_{OW} \quad (7)$$

Recent literature ⁹⁸⁻⁹ using 156 organic liquids has given, by regression, a correction to equation 7, which is given in equation 8,

$$\log WS = 0.84 - 1.18 \log_{OW} \quad (8)$$

For solids, an additional term, the energy required for the energy needed to break up the crystal structure, is required; but the influence of water in the organic phase can be neglected. This melting term was shown by Yalkowsky ¹⁰⁰ to be approximately $(0.01T_m - 0.25)$ where T_m is the melting point in degrees Celcius. Therefore for solids the predicted equation is,

$$\log WS = -0.38 - \log_{OW} - (0.01 T_m - 0.25), \quad (9)$$

and Briggs ⁹⁶ gave a correction to equation 9 using data for chemicals of known solubility, which yields equation 10,

$$\log WS = -0.01 - \log_{OW} - (0.01 T_m - 0.25), \quad (10)$$

Which indicates that the water solubility can be estimated from the octanol - water partitioning coefficient.

Organic Matter Adsorption and K_{OW}

The only additional information required to compare K_{OW} and K_{OM} (organic matter) is the concentration in soil organic matter. This has been determined for many soils, to yield an equation for various soils around the world, shown in equation 11,

$$\log K_{OM} = 0.52 \log_{OW} + 0.69 \pm 0.28. \quad (11)$$

Soil Adsorption and Water Solubility

The predicted relationship between K_{OM} and water solubility obtained from equation 4, making similar assumptions to those made in driving the K_{OW} - WS relationship, are for liquids and solids, respectively,

$$\log K_{OM} = 0.9 - 0.52 \log_{OW}, \quad (12)$$

and

$$\log K_{OM} = 0.3 - 0.52[\log WS + (0.01 T_m - 0.25)]. \quad (13)$$

Bioconcentration Factors

The distribution of a chemical between an organism and the aqueous phase around it can be regarded as a partition between the proteins and lipids in the organism and water. For substitution in equation 4, a bound water content of 0.4 g g⁻¹ for proteins ¹⁰¹ and 0.0725 M for triglycerides ¹⁰² was used.

For proteins, the calculated equation is

$$\log BCF = 0.62 \log K_{OW} + 0.46. \quad (14)$$

When the BCF for living organism was calculated a 15% protein and 5% lipid content was assumed on a fresh weight basis, BCF for protein and lipid was calculated from K_{OW}

from the appropriate equations for chemicals with and without H - bonding groups and the whole organism concentration calculated from the composition, yields for H - bonding compounds

$$\log \text{BCF} = 0.87 \log K_{\text{OW}} - 0.44, \quad (15)$$

and for hydrocarbons and chlorohydrocarbons,

$$\log \text{BCF} = 0.87 \log K_{\text{OW}} - 0.62. \quad (16)$$

In most cases, the BCF may be predicted to within one order of magnitude, giving a fairly accurate broad picture of the behaviour, even with the simplifications used in the equations.

Mass Spectra of selected Dioxins

* on selected ions indicates characteristic ions which are indicative of the different chlorinated carbon rings of PCDDs²⁷

1-chlorodibenzo-*p*-dioxin **Mass Spectrum of peak at 152**

2-chlorodibenzo-*p*-dioxin **Mass Spectrum of peak at 230**

1,2,4-trichlorodibenzo-*p*-dioxin

2,3,7-trichlorodibenzo-*p*-dioxin

1,2,3,4-tetrachlorodibenzo-*p*-dioxin **Mass Spectrum of peak at 535**

1,2,3,7/8-tetrachlorodibenzo-*p*-dioxin

1,3,6,8/7,9 - Tetrachlorodibenzo-*p*-dioxin

2,3,7,8-tetrachlorodibenzo-*p*-dioxin

1,2,3,7,8-pentachlorodibenzo-*p*-dioxin **Mass Spectrum of peak at 609**

1,2,4,7,8-pentachlorodibenzo-*p*-dioxin

1,2,4,6,7,9/6,8,9-hexachlorodibenzo-*p*-dioxin

2-nitro-1,3,4,6,7,8,9-heptachlorodibenzo-*p*-dioxin

1-nitro-3,7,8-trichlorodibenzo-*p*-dioxin

1-amino-3,7,8-trichlorodibenzo-*p*-dioxin

1-(5-bromovaleramido)-3,7,8-trichlorodibenzo-*p*-dioxin

1-(5-iodovaleramido)-3,7,8-trichlorodibenzo-*p*-dioxin

END

1 7/0 2/9 1

FIN