Dioxin and Related Compounds

Principles of Environmental Toxicology
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Learning Objectives

- Explore dioxins and dioxin-like compounds.
- Summarize the structural similarities of cogeners of dioxins and furans.
- Understand Toxicity Equivalency Factors (TEF) and Toxicity Equivalents (TEQ) for dioxins and related compounds.
- Summarize the known processes and toxicological endpoints of dioxin exposure.

Learning Objectives

- Describe the controversy and data needs concerning low-level dioxin exposure.
- Describe the observed effects and major findings of animal studies with dioxin.
- Summarize the environmental and food sources of dioxins.
- Summarize the known human risk estimations for dioxins.
- Summarize the regulatory control approaches for dioxin release.

The Organochlorine Legacy

- Halogenated organics have been used as synthetic pesticides and industrial compounds for since before WWII - stable
- Chlorinated compounds can be formed by combustion and natural processes in the presence of chlorine (dioxins)
- Often non-polar and lipophillic, they have the ability to be sequestered in fat tissue
- Can bioaccumulate up the food chain
- Can circulate in the "liposphere"

Organochlorine Compounds

- Often related to immune dysfunction, neurological effects, cancer, endocrine disruption and other toxicological endpoints
- Chlorinated compounds all around us
- Often the effects of low-level exposure are sub-clinical and "biomolecular" and this complicates the risk assessment for low-level exposure

2003 NAS Institute of Medicine Analysis

- Dioxins and Dioxin-like Compounds in the Food Supply (2003)
  - http://newton.nap.edu/catalog/10763.html

Dioxins

NAS IOM

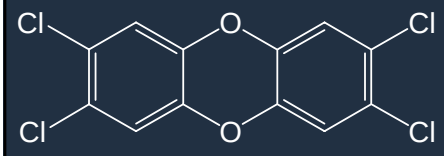
- Widespread, low-level contaminants in animal feeds and the human food supply.
- Animal fats are the primary vector of exposure.
- Dioxins metabolize slowly and accumulate in body fat over a lifetime.
- Data show decline in levels.
- Endocrine disruption is a concern.
- Exposure and children's health and development.
- High public priority to reduce dioxin levels in girls and young women.

Dioxin: Food Supply Exposure

NAS IOM

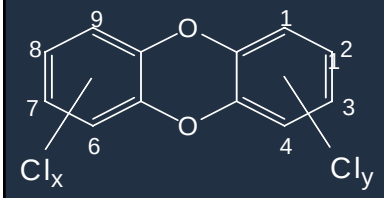
- Animal production systems
  - Airborne deposition on grazing areas or water bodies
  - Geographic variability due to sources (incineration)
- Human foods
  - Relatively uniform exposure due to food distribution patterns
- Food-consumption patterns
  - High fat diets = higher exposure
  - Animal fats, full-fat dairy, fatty fish

Chlorinated Dibenzo Dioxins

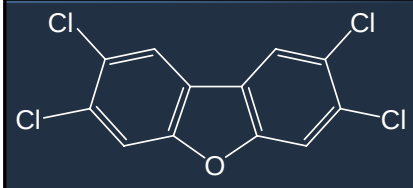


2,3,7,8-Tetrachlorodibenzo-p-dioxin
TCDD

PCDDs

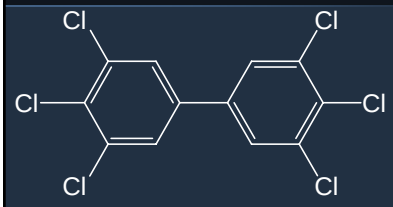


Chlorinated Dibenzo Furans



2,3,7,8-Tetrachlorodibenzo-furan

Polychlorinated Biphenyls



3,3',4,4',5,5'- Hexachlorobiphenyl

Background

- 75 dioxin cogeners and 135 dibenzofuran congeners.
- In general, CDD's and CDF's are present in human adipose tissue and fish and bird samples at a sub - μg/kg level.
  - Many of these being the less or non-toxic isomers.
- In general, relative toxicity:
- CCD > CDF >> PCB >> CN

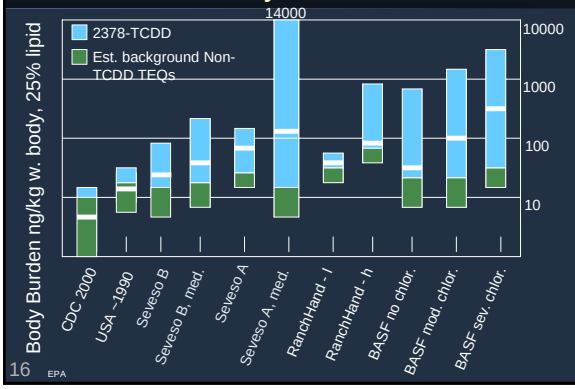
Combining Risks from Dioxins

- Dioxins share a "common mechanism of toxicity".
- Toxicity Equivalency Factors (TEF) compare the toxicity of different dioxins.
- TEF are expressed in terms of Toxicity Equivalents (TEQ).
- TEQ is the amount of TCDD it would take to equal the combined toxic effect of all the dioxins found in that mixture.

The TEF Scheme for TEQ_{DF}

Furan (F) congener	TEF	Dioxin (D) congener	TEF
2,3,7,8-TCDF	0.1	2,3,7,8-TCDD	1.0
1,2,3,7,8-PeCDF	0.05	1,2,3,7,8-PeCDD	0.5
2,3,4,7,8-PeCDF	0.5	1,2,3,4,7,8-HxCDD	0.1
1,2,3,4,7,8-HxCDF	0.1	1,2,3,6,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDF	0.1	1,2,3,7,8,9-HxCDD	0.1
1,2,3,7,8,9-HxCDF	0.1	1,2,3,4,6,7,8-HpCDD	.01
2,3,4,6,7,8-HxCDF	0.1	1,2,3,4,6,7,8,9-OCDD	.001
1,2,3,4,6,7,8-HpCDF	0.01		
1,2,3,4,7,8,9-HpCDF	0.01		
1,2,3,4,6,7,8,9-OCDF	0.001		

Dioxin Body Burden Levels



Dioxin Exposure Case Studies

- Love Canal (1940s-1950s).
  - Hazardous waste landfill release.
- Times Beach (pre-1982).
  - Chemical mix used to oil streets.
- Agent Orange.
  - Vietnam "Operation Ranch Hand".
- Seveso, Italy (1976).
  - 2,4,5 Trichlorophenol industrial accident.
- BASF/IB (1953, other).
  - Chlorinated herbicide manufacturing workers.

Background Serum, US 95-97

	TEQ_{DFP} (pg/g lipid)	**2,3,7,8-TCDD** (pg/g lipid)
Median	18.7	1.9
Mean	22.1	2.1
95th Percentile	38.8	4.2

Adult background intake estimate 70 pg TEQ_{DF}/d

Dioxin Toxicity

- TCDD characterized as a "human carcinogen"
  - Other dioxins characterized as "likely human carcinogens".
- Dioxins can alter the fundamental growth and development of cells.
- Impact of dioxins on cells results in:
  - Adverse effects upon reproduction and development.
  - Suppression of the immune system.
  - Chloracne (a severe acne-like condition).

Acute Dioxin Poisoning: Chloracne

Ukrainian President Viktor Yushchenko
Dioxin Poisoning

Dioxin Exposure

- Dioxins are highly persistent and can bioaccumulate.
- 95% of dioxin intake for a typical person comes through dietary intake of animal fats.
- Low exposure:
  - Breathing air containing trace amount of dioxins.
  - Ingestion of soil containing dioxins.
  - Absorption through skin contacting air, soil, or water containing minute levels.

Dioxin Exposure, 2

- Environmental processes result in widespread, low-level exposure of the general population.
- Dioxin levels in the environment have declined since the 1970s.
- Dioxin emissions in the US decreased by ~80% between 1987 and 1995.

General Population Body Burden

- US CDD/CDF range = 8.5 pg TEQ/g lipid to 50.0 pg $TEQ_{DF\text{-}WHO98}$/g lipid
- Mean 21.1 pg $TEQ_{DF\text{-}WHO98}$/g lipid

General Population Intake

- US CDD/CDF estimate 41 pg $TEQ_{DF\text{-}WHO98}$/d or 0.59 pg $TEQ_{DF\text{-}WHO98}$/kg/d
- US CDD/CDF/PCB estimate 65 pg $TEQ_{DF\text{-}WHO98}$/d or 1 pg $TEQ_{DF\text{-}WHO98}$/kg/d
- Children: US CDD/CDF estimate 54 pg $TEQ_{DF\text{-}WHO98}$/d or 3.6 pg $TEQ_{DF\text{-}WHO98}$/kg/d
  - Decrease with age
- 5 compounds = 70% load
  - TCDD, PeCCD, PeCDF HxCDF, PCB 126

Dioxin Effects in Humans

- The amount of dioxin found in the tissues of the general human population (Body Burden) approaches (w/in a factor of 10) the levels at which adverse effects occur.
- Despite which, there is no clear indication of increased disease in the general population.
  - Limitation of current data and scientific tools.

Dioxin Effects in Humans

- 1 in 100 to 1 in 1,000 increased chance of experiencing cancer related to dioxin exposure in the general population.
- Cancer risk in 2000 analysis indicates about 10-fold higher chance than estimated in 1994 reassessment.

Children and Concern Groups

- Fetuses, infants, and children may be more sensitive to dioxin exposure because of rapid growth.
  - Data on risks to children is limited.
- U.S. Air Force personnel exposed to Agent Orange during the Vietnam War.
- Other populations have experienced elevated exposure from:
  - Industrial accidents.
  - Unusually high consumption of fish, meat and dairy products.

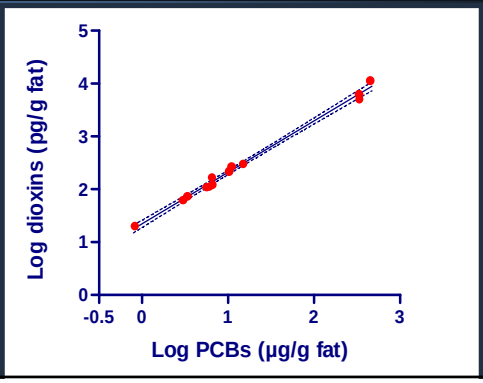
Dioxin Effect Controversy

- Enzyme induction and indicators of altered cellular function may not clearly indicate toxic response.
- Changes in biology and biochemistry from low-exposure:
  - Adaptive (w/ little or no adverse impact).
  - Adverse(?).

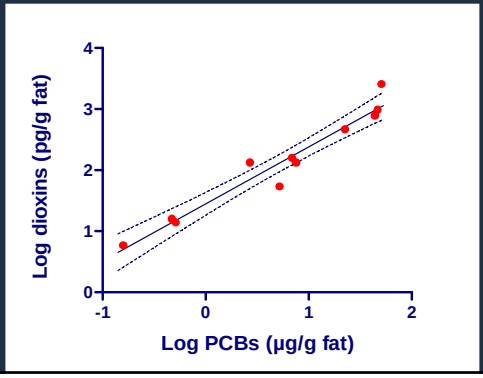
Case Study: Belgium 1999

- Transformer oil added to animal feed at feed mills.
- Poultry: reduction in egg hatchability, reduced weight gain, an increased mortality, edema, ataxia.
- PCBs and dioxins in animals products.
- 60,000,000 kg of animals destroyed.
- Meat product embargo.

Belgium: Dioxins and PCBs in Feedstuffs



Belgium: Dioxins and PCBs in Chicken



Clinicopathologic Concepts

- Syndrome induced by CDDs in a given species of animal is comparable to that induced by CDFs, PCBs, PBBs, CNs.
- Pathogenesis of the disease is the same – suggests that these chemicals involve the same receptors.
  - Typical exposure may be a mixture of isomers and compounds.
  - Best to view the disease syndrome in terms of etiology rather than specific insult.

Clinicopathologic Syndrome

- Varies from animal species to animal species.
- Skin of primates, rabbits (ears), cattle & some mice show characteristic follicular dermatitis.
  - Chloracne: visible and reversible lesion.
- Livers of chickens, rabbits (mice) show necrotic response of lethal severity.
  - Guinea pigs, cattle, NH primates: enlarged liver, epithilial hyperplasia of bile duct/gall bladder.
- Some animals show epithilial lesions: GIT, renal.

Clinicopathologic Syndrome

- The one organ that uniformly shows lesions in all species is the thymus. Banbury
  - Often weighs 25% less in lethal intoxications.
    - Site of early life formation of lymphocytes and a site of antibody production.
- Severe intoxication in birds accompanied by fluid accumulation (chick edema).
- Interesting feature:
  - Total dose of TCDD required to produce disease is less if the dose is spread over time compared to a single dose.

LD_{50}

Specie	LD_{50} TCDD (µg/kg)
Pig (most sensitive)	0.6
Avian (very sensitive)	No exact
Rat	22-45
C57bl Mice	114-284
Monkey R.	<70
Rabbit N.Z.	115
Hamsters	5050

Observations

- In general, young animals and females may be more susceptible to intoxication (field).
  - Not observed in lab studies.
- Neonatal death, poor survival of young, female infertility and reproductive failure are indicators of field problems.
- At lethal dose levels, the time between exposure and death is unusually long.
  - Guinea pig, rat, mice: 2-3 wks.
  - Monkeys: 6 wks.

Observations

- Except for animals with severe liver necrosis (chickens, rabbits), cause of death not usually attributed to a specific organ or system pathology.
- In general, animals exhibit wasting disease.
  - Resembles starvation, anorexia.
- In environmental exposures, the disease is complicated by opportunistic infection.

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Metabolism of TCDD

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- Dog and rat studies.
- Major metabolites are hydroxylated compounds.
- Most is eliminated as parent compound in feces.
- Chronic rodent bioassays, life-term and short duration have addressed the issues of tumor initiation, promotion, co-carcinogenesis, DNA interaction, mutagenesis and clastogenesis.

Carcinogenicity - Mutagenicity

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Animal	Dosage TCDD μg/kg/d	Response
Rat SD	0.1	Hepatocellular, squam. carc.
Rat SD	0.01	Hepatocellular nodules
Rat SD	0.001	No ↑ in tumors
Mouse B6	0.3	Hepatocellular, thyroid tumors
Mouse B6	0.07	Hepatocellular tumors
Mouse B6	0.03	No ↑ in tumors

Suggested Mechanisms

- Toxicity and carcinogenicity.
  - Alteration of cell membrane function and cell-cell communication.
  - Effect on Vitamin A function.
  - Membrane lipid peroxidation.
  - Thyroid hormones.
  - Hormonal alterations.
  - DNA modifications.

 Banbury

Hepatotoxicity Mechanisms

- Experiments suggest O_2• (superoxide) formation and initiation of peroxidation by Fe^{2+}.
  - Progressive liver damage.
- TCDD inhibits hepatic Se-GSHpx and reduced glutathione.
  - Good correlation of GSHpx activity and survival.
  - Lipid peroxidation endpoint.

 Banbury

Dioxin: Early Molecular Events

EPA

1. Diffusion into the cell.
2. Binding of the AhR protein.
3. Dissociation from hsp90.
4. Active translocation from cytoplasm.
5. Association with Arnt protein.
6. Conversion of liganded receptor heteromer to enhancer DNA.
7. Enhancer activation.
8. Altered DNA configuration.
9. Histone modification.
10. Recruitment of additional protein.
11. Nucleosome disruption.
12. Increased accessibility of transcriptional promoter.
13. Binding of transcription factors to promoter.
14. Enhanced mRNA and protein synthesis.

Effects of TCDD and Related Compounds

Effect	Human	Monkey	Rat	Fish	Avian wildlife	Marine mammals
Enzyme induction	+	+	+	+	+	+
Acute lethality	0	+	+	+	+	+
Wasting syndrome		+	+	+	+	+
Teratogenesis, mortality	+/-	+	+	+	+	+
Endocrine effects	+/-	+	+	+	+	+
Immunotoxicity	+/-	+	+	+		+
Carcinogenicity	+/-		+	+		
Neurotoxicity	+	+	+			
Porphyria	+	0	+			
Hepatotoxicity	+	+	+	+	+	+

 EPA

Environmental Source Types

- Combustion and incineration sources.
- Metals smelting, refining and processing.
- Chemical manufacturing/processing.
- Reservoir sources (e.g. soils).
- Biological and photochemical processes.
- Significant regulatory pressure to limit release.

 EPA

TEQ_{DF} Releases - Air_{US}

EPA

(g TEQ/yr) Incineration	1995	1987
Municipal waste	1250	8877
Medical waste/path.	488	2590
Forest, brush, and straw fires	208	170
Cement kilns (HW)	156	118
Sewage sludge	14.8	6.1
Crematoria	9.1	5.5
Hazardous	5.8	5
Tire combustion	0.11	0.11
Cigarettes	0.8	1

TEQ_{DF} Releases – Air_{US}, 2

EPA

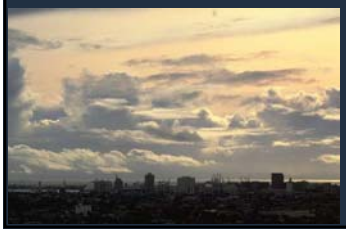
(g TEQ/yr) Power/Energy Generation		1995	1987
Vehicle fuel	-leaded	2	37.5
	-unleaded	5.9	3.6
	-diesel	35.5	27.8
Wood comb.	-resident	62.8	89.6
	-industrial	27.6	26.4
Coal comb.	-utility	60.1	50.8
Oil comb.	-ind'st./utility	10.7	17.8

TEQ_{DF} Releases – Air_{US}, 3

Releases (g TEQ/yr) to air

EPA

	1995	1987
Total quantified releases to air	2700	13100



TEQ_{DF} Releases – $Water_{US}$

Releases (g TEQ/yr) to water

EPA

	1995	1987
Bleached wood pulp and paper mills	19.5	356
Total quantified releases to water	19.9	356

TEQ_{DF} Releases – $Land_{US}$

Releases (g TEQ/yr) to land

	1995	1987
Bleached wood pulp and paper mill sludge	1.4	14.1
Municipal wastewater treatment sludge	76.6	76.6
Commercially marketed sewage sludge	2.6	2.6
2,4-Dichlorophenoxy acetic acid	28.9	33.4
Total quantified releases to land	110	127

EPA

TEQ_{DF} Releases – $Overall_{US}$

	1995	1987
Overall quantified releases to the open and circulating environment (g TEQ/yr)	2830	13560

EPA

AP

Unquantified Sources

Category	Unquantified sources
Combustion sources	Uncontrolled combustion of PCBs Agricultural burning
Metal smelting and refining	Primary Al, Mg, Ni
Chemical Manufacturing	Mono- to tetrachlorophenols Pentachlorophenol Chlorobenzenes Chlorobiphenyls (leaks/spills) Dioxazine dyes and pigments 2,4-D Tall oil-based liquid soaps
Biological and photochemical processes	Composting
Reservoir sources (runoff, erosion)	Air, Sediments, Water, Biota PCP-treated wood

 EPA

Source Release Reduction

- 80% decrease between 1987 and 1995 of dioxin and CDDs/CDFs to air, water and land. EPA
  - Due to reduction in air emissions from municipal and medical waste incinerators.
  - Regulations promulgated in 1995 for municipal waste combustors and in 1997 for medical waste incinerators should result in greater than 95% reduction in dioxin emissions from these two categories.

Control Efforts for Air

- The Clean Air Act (CAA) and its amendments requires emission limits based on “maximum achievable control technology” (MACT). EPA
  - Changes in 1995 for municipal waste and 1997 for medical waste incinerators should result in greater than 95% reduction in dioxin emissions.
- CAA and the Resources Conservation and Recovery Act (RCRA) authorize the regulation emissions from facilities that burn HW.

Control Efforts for Water

- The Clean Water Act (CWA) manages releases through risk-based and technology-based tools. EPA
  - 1984 ambient water quality for 2,3,7,8-TCDD – a guidance for state water quality criteria.
- National Pollutant Discharge Elimination System (NPDES) regulates discharge based on state ambient water quality.

Control Efforts for Water, 2

- Pulp and paper facilities were the largest known industrial dischargers of dioxin into water.
  - 1998 CWA guidelines will reduce dioxin discharge from pulp and paper facilities by at least 96%.
- NPDES will places stringent performance requirements through combination of technology-based, health-based and state water quality standards.

Control Efforts for Water, 3

- 1992 maximum contaminant level goal (MCLG, a non-enforceable,voluntary health goal) of zero.
- Safe Drinking Water Act (SDWA) enforces a maximum contaminant level (MCL) of $3x10^{-8}$ mg/l for TCDD.

Control Efforts for Land

- Superfund and RCRA Corrective Action programs for dioxin (Times Beach and Love Canal).
- Hazardous Waste Identification and Disposal Rules under RCRA designed to prevent future contamination.
- The Toxic Substance Control Act (TSCA) authorizes restricted use of dioxin – contaminated pulp and paper sludge.
- 1999 regulations limit dioxin content of cement kilns and sludge from POST facilities when by-product material is used as soil additives.

Control Efforts for Products

- The Federal Insecticide Fungicide and Rodenticide Act (FIFRA) and TSCA authorizes control or elimination of certain chemicals.
  - 2,4,5-T and pentachlorophenol (PCP).

Environmental Media

Media	TEQ_{DF} concentrations
Rural soils	1-6 pg/g (ppt)
Urban soils	7-20 pg/g
Sediments	1-60 pg/g
Rural air	0.002-0.02 pg/m^3
Urban air	0.02-0.2 pg/m^3

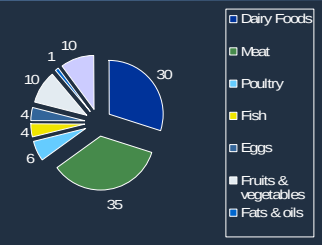
EPA

Estimate Levels in Food

Food type	Total (pg TEQ_{DF}/g fresh weight)
Beef	0.29
Pork	0.31
Eggs	0.13
Milk	0.047
Dairy products	0.18
Marine fish	0.61
Freshwater fish	2.4
Marine shellfish	0.83
Vegetable fats	0.093
Water	NA

EPA

% Contribution of Food Dioxin Intake Children 1-5 Yrs



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Background/Body Burden Changes

- Body burdens late 1980s
  30 – 80 pg TEQ/g lipid (30 – 80 ppt) EPA
  - Midpoint of ~55 pg TEQ/g lipid including all dioxins, furans, and dioxin-like PCBs.
- High-end estimates (~ 1% of general pop.) may be 3 times higher.
  - Based on blood-level data and consumption of fat as surrogate for dioxin intake.
- CDD/CDF/PCB body burden in late 1990s
  25 ppt (TEQ, lipid basis).

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Risk

- Receptor binding and most early biochemical events are likely to demonstrate low-dose linearity.
  - If findings imply low-dose linearity in biologically-based cancer models, then the probability of cancer risk will be linearly related to exposure to TCDD at low doses.
- Until the mechanistic relationships are better understood, the shape of the dose-response curve for risk can only be inferred with uncertainty.

63 EPA