Dioxin and Related Compounds in the Human Food Chain

Food Toxicology
Instructor: Gregory Möller, Ph.D.
University of Idaho

Learning Objectives

- Explore dioxins and dioxin-like compounds in the food supply
- 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

- 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

- 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
PCDDs
Chlorinated Dibenzo Furans
Polychlorinated Biphenyls
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}
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

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

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.
  - 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} 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.

Metabolism of TCDD

- 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 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.

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.

Early Molecular Events

- Diffusion into the cell.
- Binding of the AhR protein.
- Dissociation from hsp90.
- Active translocation from cytoplasm.
- Association with Arnt protein.
- Conversion of liganded receptor heteromer to enhancer DNA.
- Enhancer activation.
- Altered DNA configuration.
- Histone modification.
- Recruitment of additional protein.
- Nucleosome disruption.
- Increased accessibility of transcriptional promoter.
- Binding of transcription factors to promoter.
- Enhanced mRNA and protein synthesis.

Effects of TCDD and Related Compounds

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.

TEQ_{DF} Releases - Air_{US}

TEQ_{DF} Releases – Air_{US}, 2

TEQ_{DF} Releases – Air_{US}, 3

TEQ_{DF} Releases – $Water_{US}$

TEQ_{DF} Releases – $Land_{US}$

TEQ_{DF} Releases – $Overall_{US}$

Unquantified Sources

Source Release Reduction

- 80% decrease between 1987 and 1995 of dioxin and CDDs/CDFs to air, water and land.
  - 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).
  - 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.
  - 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
Estimate Levels in Food
% Contribution of Food Dioxin Intake Children 1-5 Yrs
Background/Body Burden Changes

- Body burdens late 1980s
  30 – 80 pg TEQ/g lipid (30 – 80 ppt)
  - 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).

Risk

- Receptor binding and most early biochemical events are <u>likely</u> to demonstrate low-dose linearity.
  - <u>If</u> 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 <u>inferred with uncertainty</u>.