DIOXIN ANALYSES FOR ECOLOGICAL RISK ASSESSMENT: SCIENTIFIC APPROACH AND PRACTICAL CONSIDERATIONS

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ABSTRACT

Successful evaluation of excessive risks to health caused by exposure to Ah-agonistic dioxins and related congeners (halogenated furans and co-planer PCBs) depends upon important biological and analytical factors. Consideration of these factors is essential whenever one evaluates risks posed by a class of potent chemicals with varying degrees of related toxicity (e.g., PAHs or PCBs). Use of EPA Biological Technical Assistance Groups and outside experts on problems with complex stressors, such as dioxins, helps to guide effective sampling and analyses that can address both remediation and restoration concerns for contaminated sites. Potent toxicants, like 2,3,7,8-substituted halogenated dioxins and furans, challenge current analytical methods= capabilities for accuracy, precision, and low method detection limits with good QA/QC. To statistically quantitate exposures and potential risks to wildlife, method quantitation limits (MQL) should always aim to be about 2- to 10-fold lower than risk-based concentrations in the media of concern. For 2,3,7,8-TCDD, the MQL often needs to be near or less than 1 ppt (pg/g) in samples. Various field and laboratory analytical methods have advantages and disadvantages for certain applications; generally, use of bioassays with sensitive linear responses for total TCDD-TEQ (toxic equivalent) determinations, and instrumental GC/MS for specific congener determinations, are currently preferred. TEFs (toxicity equivalent factors) normalize Ah-agonist congeners to TEQs of 2,3,7,8-TCDD; use of WHO TEFs for terrestrial screening, and derivation of specific toxicity potency factors for more quantitative terrestrial and aquatic risk assessments, are recommended. Congener pattern analysis is sometimes indicated to help determine probable sources of Ah-agonists based on fingerprints of approximately 21 of the most potent chlorinated congeners. Sampling designs must be representative of receptors= exposure units (e.g., home-ranges), and suitable reference areas are essential for discerning site-related sources and concentration gradients from prevalent natural background contamination.

INTRODUCTION

Dioxins, and related chemical congeners with Ah-receptor agonist activity, are exemplified by the most potent known molecular form: 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD). These chemicals react with differing affinities for the cellular arylhydrocarbon (Ah) receptor to begin a cascade of biochemical events that often lead to toxicity. Dioxin congeners with Ah-agonist activity comprise a complex mixture of variably toxic contaminants that pose a considerable challenge for conducting accurate quantitative risk assessments. Part of the challenge is common for all potent toxicants: achieving adequately low analytical detection limits in biotic and abiotic samples with trace analytical methods. Another difficulty is estimating the total toxicologic risk posed by a complex mixture of chemicals. The chemical mixture profile in the environmental abiotic media is often not the same as that observed in the biotic receptor tissue of concern, and antagonistic or synergistic co-contaminants may be present. Biological factors can affect dioxin toxicity, such as: bioaccessibility of dioxin congeners in various media, different toxicokinetics (absorption, distribution, metabolism, and elimination) and toxicodynamics (dose-response curves at differing target tissues), varied exposure duration and frequency, and extrapolations of toxic endpoints and severity of effects between species. Another complication for risk assessments is that Ah-agonistic dioxins and congeners are ubiquitous in the environment, which makes the determination of releases from point sources and measuring their gradients of concentrations to background levels very difficult. This paper outlines some of the major issues and shows examples of current approaches for assessing quantitative risks of mixtures of Ah-agonistic dioxins and congeners (dioxins).

PLANNING for Dioxin Risk Assessments

- Consider Risk Management Goals and Framework for ecological risk assessment
- Employ Biological Technical Assistance Groups (BTAGs) with expert consultants
- Develop Quality Assurance Project Plans (QAPPs) with risk management input
- Prepare Sampling and Analysis Plans (SAPs) to assess complex dioxin mixtures
  - Sample representatively of major exposure pathways
  - Sample gradients of contamination with adequate density
  - Use Data Quality Objectives to incorporate sound design and QA/QC
  - Sample and compare results with background levels from reference areas
- Anticipate interpretation of results
  - Establish decision criteria for adequacy of sampling and results: iterations? proxies?
  - Produce toxicity reference values (TRV) with risk-based concentrations (RBC)

ANALYTICAL CHALLENGES FOR 2378-TCDD AND AH-AGONIST CONGENERS

Quality Assurance and Quality Control requirements

- **Field samples**: ≥5% rate
  - Blanks: to help assure absence of cross-contamination
  - Replicates: to assess precision through processing
  - Spikes: low, medium, and high standards for accuracy

- **Lab samples**: ≥5% rate
  - PARCC: generally the usual requirements (precision, accuracy, representativeness, completeness, consistency)
  - Matrices: extraction efficiency and recovery needs to be adequate for tissues
  - Detections: Method Quantitation Limit (MQL) < 2-10x risk-based concentrations

Typical PCDD/F-PCB Target Analytes And Minimum Detection Levels

Target analyte		Water (pg/L)	Solid (pg/g)
2,3,7,8-	TCDF	10	1
2,3,7,8-	TCDD	10	1
1,2,3,7,8-	PeCDF	50	5
2,3,4,7,8-	PeCDF	50	5
1,2,3,7,8-	PeCDD	50	5
1,2,3,4,7,8-	HxCDF	50	5
1,2,3,6,7,8-	HxCDF	50	5
2,3,4,6,7,8-	HxCDF	50	5
1,2,3,7,8,9-	HxCDF	50	5
1,2,3,4,7,8-	HxCDD	50	5
1,2,3,6,7,8-	HxCDD	50	5
1,2,3,7,8,9-	HxCDD	50	5
1,2,3,4,6,7,8-	HpCDF	50	5
1,2,3,4,7,8,9-	HpCDF	50	5
1,2,3,4,6,7,8-	HpCDD	50	5
	OCDD/F	100	10
77-TCB		5	0.5
105-PeCB		60	6
114-PeCB		600	60
118-PeCB		60	6
123-PeCB		40	4
126-PeCB		40	4
156-HxCB		60	6
157-HxCB		60	6
167-HxCB		60	6
169-HxCB		60	6
170-HpCB		60	6
180-HpCB		60	6
189-HpCB		60	6

*Based on the lowest calibration standard specified in the EPA Methods 1613B and Draft 1668), 10-g sample size for solids, and 1 L for water with 20-uL final volume.

METHODS TO ESTIMATE TOTAL DIOXIN AND CONGENER AH-AGONIST ACTIVTY

(TEQ = Total Equivalents of 2378-TCDD Activity)

In vitro Bioassays for Ah-Agonist Activity: measures TEQ

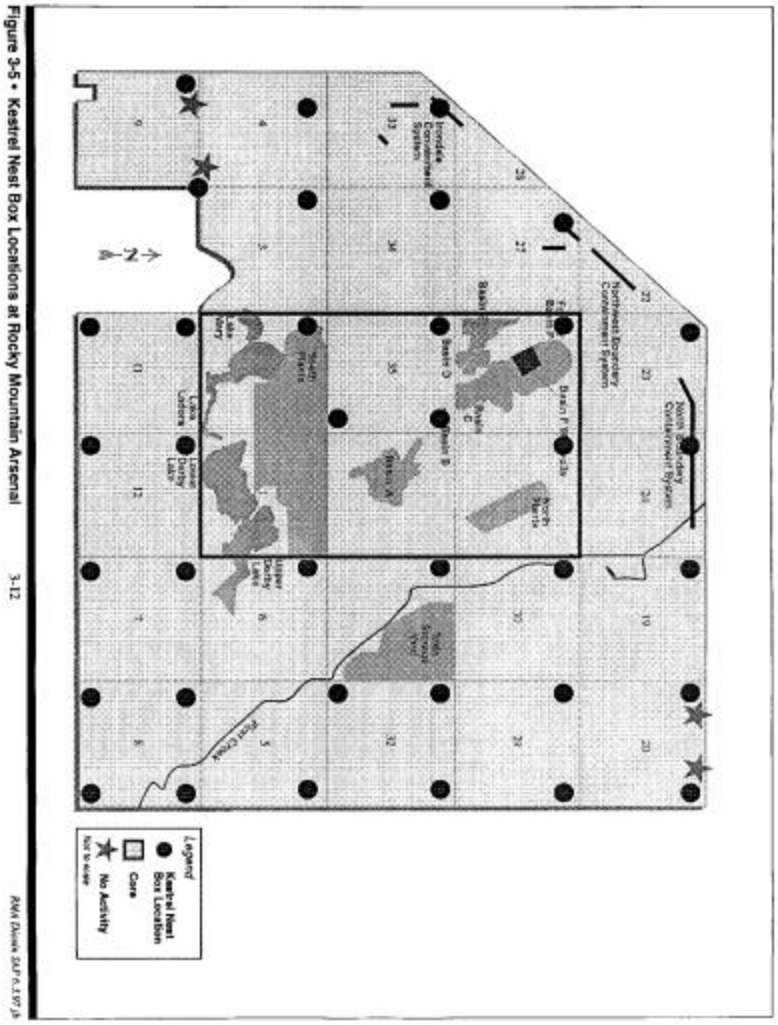
- Strengths: measures total Ah-agonist activity of mixture, compared to calculated TEQ
- Weaknesses: over-estimates bioavailability of Ah-agonists to target cell compared to *in vivo*
  "biomarker" (EROD, Luciferase) activity is not always related to toxic response
  competitive inhibition or synergism is hidden, but net result is measured

Congener analyses for Ah-Agonist Concentrations: calculates TEQ

- Strengths: measure specific chemical contributors to Ah-agonist activity
- Weaknesses: trace method MDL problems gives uncertainty about low quantitations
  multiplying concentrations by uncertain TEFs creates uncertain TEQ results
  may miss some inhibitory or synergistic effects by mixtures on TEQs

WHO CONSENSUS TEFs FOR MAMMALS, FISH, AND BIRDS

PCDD/F and PCB Ah-agonist Congeners		**HUMANS/ MAMMALS**	**FISH**	**BIRDS**
2,3,7,8-	TCDD	1	1	1
1,2,3,7,8-	PeCDD	1	1	1
1,2,3,4,7,8-	HxCDD	0.1	0.5	0.05
1,2,3,6,7,8-	HxCDD	0.1	0.01	0.01
1,2,3,7,8,9-	HxCDD	0.1	0.01	0.1
1,2,3,4,6,7,8-	HpCDD	0.01	0.001	<0.001
	OCDD	0.0001	-	-
2,3,7,8-	TCDF	0.1	0.05	1
1 ,2,3,7,8-	PeCDF	0.05	0.05	0.1
2,3,4,7,8-	PeCDF	0.5	0.5	1
1,2,3,4,7,8-	HxCDF	0.1	0.1	0.1
1,2,3,6,7,8-	HxCDF	0.1	0.1	0.1
1,2,3,7,8,9-	HxCDF	0.1	0.1	0.1
2,3,4,6,7,8-	HxCDF	0.1	0.1	0.1
1,2,3,4,6,7,8-	HpCDF	0.01	0.01	0.01
1,2,3,4,7,8,9-	HpCDF	0.01	0.01	0.01
	OCDF	0.0001	0.0001	0.0001
3,4,4',5-	TCB (81)	0.0001	0.0005	0.1
3,3',4,4'-	TCB (77)	0.0001	0.0001	0.05
3,3',4,4',5-	PeCB (126)	0.1	0.005	0.1
3,3',4,4',5,5'-	HxCB (169)	0.01	0.00005	0.001
2,3,3',4,4'-	PeCB (105)	0.0001	<0.000005	0.0001
2,3,4,4',5-	PeCB (114)	0.0005	<0.000005	0.0001
2,3',4,4',5-	PeCB (118)	0.0001	<0.000005	0.00001
2',3,4,4',5-	PeCB (123)	0.0001	<0.000005	0.00001
2,3,3'4,4',5-	HxCB (156)	0.0005	<0.000005	0.0001
2,3,3',4,4',5'-	HxCB (157)	0.0005	<0.000005	0.0001
2,3',4,4',5,5'-	HxCB (167)	0.00001	<0.000005	0.00001
2,3,3',4,4',5,5'-	HpCB (189)	0.0001	<0.000005	0.00001



EXAMPLE OF SITE-SPECIFIC APPROACH TO ASSESS DIOXINS

- **Rocky Mountain Arsenal (RMA), Denver, CO**
  - 27 square miles, short-grass prairie habitat, future USFWS refuge
  - uncertainty as to sources or releases of dioxins, from former pesticide manufacturing
  - risk managers wish to know if any "incremental risk" is added to that posed by dieldrin

- **Tier I = Exposure Study**
  - let biota "sample" larger scale areas of the RMA for integrated exposure to dioxins
  - stratified sampled tissues, on- and off-site =

    American kestrels: eggs
    Great Horned Owl: livers (*See Map*)
    Carp: eggs
  - both bioassay (H4IIE-luciferase) and instrumental congener analyses to be performed
  - evaluate 3 sets of results:

    TEQ measured by bioassay
    TEQ calculated by TEFs, proxies for non-detects = ½ MDL
    Congener patterns by principle components analysis
  - compare results with off-site reference tissues, to determine if on-site source exists

- **Tier II = Nature and Extent, and Toxicity Studies**

INTERPRETATION OF BIOMONITORING RESULTS FOR DIOXINS AT RMA

Outcome 1: There are no significant elevations for on-site compared to off-site dioxin levels

- calculate any incremental risk posed by background levels of dioxin in regards to dieldrin

Outcome 2: Uncertain but possible elevations of dioxins may exist between on- and off-site

- consider further tiers or iterations of sampling to reduce uncertainty of exposures, possibly use other species with smaller exposure ranges for better refinement

Outcome 3: Significant elevations of dioxin exist on-site compared to off-site

- sample further to determine nature and extent of dioxins, and major pathways of exposure, sample abiotic + biotic media, use species with smaller home ranges for better refinement
- calculate inherent and additive risks to exposed receptors, using initial TRVs in tissues

ESTIMATING RISKS TO RECEPTORS EXPOSED TO DIOXINS AT RMA

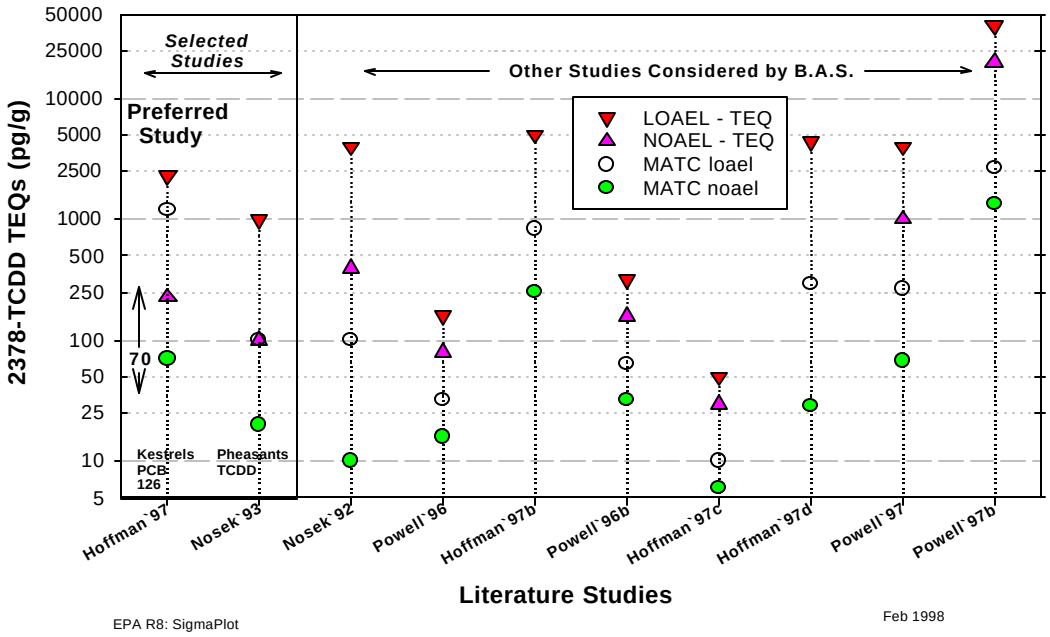
Toxicity Reference Values (TRVs) were derived from literature studies

- Maximum Allowable Tissue Concentrations (MATCs) for sampled tissues
- Both NOAEL and LOAEL benchmarks identified for toxic (adverse) endpoints
- Extrapolation performed to "normalize" study results for: duration, severity, and endpoint

Example of Literature Reviewed for Kestrel Egg Residues of Dioxin Congeners and Toxicity

- RMA Biological Assessment Subcommittee (B.A.S.) reviewed toxicological studies
- Criteria were used to elevate the strongest candidate studies for primary consideration
  - a "preferred" study was identified from eligible "selected" studies (*see Chart*)
  - MATCs represent "normalized" extrapolated values from literature NOAELs/LOAELs
- A tabulated "range" of MATC values were derived to use at RMA for estimating dioxin risks
  - TEF ranges from RPF (Relative Potency Factors, WHO 1998)
  - single MATCs derived from WHO TEF

Kestrel Egg Toxicity Data for TCDD MATCs at RMA



PROPOSED MATCS FOR TCDD AS TOXIC EQUIVALENTS (TEQ) IN BIOTA AT RMA

Species of Concern	MATC $_{NOAEL-Based}$	MATC $_{LOAEL-Based}$	Study
* *American kestrel eggs*	**70 pg/g TCDD-TEQ** (TEF-based range: 50-260 pg/g)	1,200 pg/g TCDD-TEQ (TEF range: 800 - 4,600 pg/g)	Hoffman et al. 1997
American kestrel eggs	**20 pg/g TCDD-TEQ** (TEF-based range: 14-80 pg/g)	100 pg/g TCDD-TEQ (TEF range: 70-400 pg/g)	Nosek et al. 1993
American kestrel eggs	**20 ppb TCDD-TEQ** (TEF range: 12-70 pg/g)	400 pg/g TCDD-TEQ (TEF range: 280-1,600 pg/g)	Powell et al. 1997
American kestrel eggs	**15 ppb TCDD-TEQ** (TEF-based range: 8-30 pg/g)	**Not available**	Elliott et al. 1998
**** G H Owl liver:*** nestling tox.	**210 pg/g TCDD-TEQ** (TEF range: 150 - 800 pg/g)	800 pg/g TCDD-TEQ (TEF range, 500 - 3,000 pg/g)	Hoffman et al. 1996b
* ***G H Owl liver***: adults reproductive	**14 pg/g TCDD-TEQ** (liver:egg ratio and TEF-range: 7 - 60 pg/g)	230 pg/g TCDD-TEQ (liver:egg ratio, TEF range: 120-920 pg/g)	Hoffman et al. 1997
**** Northern pike eggs***	**1,200 pg/g TCDD-TEQ**	1,800 pg/g TCDD-TEQ	Elonen et al. 1997
****Other fish spps. Eggs***	**170 - 500 pg/g TCDD-TEQ**	400 - 1,100 pg/g TCDD-TEQ	Henry 1997 (noael); Elonen et al. 1997

***** Studies selected as the most applicable for RMA quantitative analysis.

CONCLUSIONS

1. Wildlife can serve as "biological monitors" for integrating scales of exposure to dioxins
   - Representative sampling is needed on-site, reference samples delineate background
   - Proper tissues that bioaccumulate and/or are target tissues should be analyzed
   - Deriving TRVs can help ensure that target analytical MDLs are met below RBCs

2. Both bioassays for measured TEQ & instrumental analyses for calculated TEQ have merit
   - *In vitro* bioassays measure total TEQ, but in cell cultures and with possible interactions
   - Congener analyses measure concentrations and patterns; non-detects are problems

3. Toxicity reference values (TRVs) can be derived, and new WHO TEFs or RPFs applied
   - Receptor and endpoint specific benchmarks can be used for Hazard Quotient analyses
   - Risk-based concentrations can be derived to help determine adequately low MDLs
   - EPA set an interim action level for soil at 1 ppb TCDD, which may not protect wildlife

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