Reference Dose (RfD) for a Complex Mixture of Methylmercury, PCBs, and DDT: Implications for Risk Assessment

Kenneth A. Poirier, Michael L. Dourson, and Qiyu Zhao

Toxicology Excellence for Risk Assessment

Faroe Islands Mixture Exposure

- Inhabitants have a very unique toxic chemical exposure profile.
- Seafood is a main food source - fish (44%) and pilot whale (9.5%) daily consumption.
- Contaminated not only with MeHg, but also with PCBs and DDT.

Faroe Islands Mixture Exposure II

- These three chemicals together present a mixture exposure in the FI.
- Unique opportunity to study the adverse health effects following exposure to a chemical mixture in human population.

USEPA Mixtures Guidelines

- EPA (1986) published a guideline for conducting risk assessment of chemical mixtures. Recently, an updated guideline (USEPA, 1999) has been published.
- Two types of methods for a mixture assessment based on whether data available is on whole mixture or mixture components.

USEPA Mixtures Guidelines II

- Type I - whole mixture data based on the mixture of concern or that of a similar mixture.
- Type II - component data based on interaction data, a similar mode of action, or if the components can be grouped into a chemical class.

Faroe Islands Mixture and EPA Methods

- For FI, data are available for both whole mixture and mixture components.
- Recently published studies on developmental neurobehavioral effect in children born to mothers living on the islands might be useful in directly deriving a mixture RfD.

Faroe Islands Mixture and EPA Methods II

- In addition, EPA established RfDs for exposures to MeHg, PCBs and DDT and their corresponding toxicological data evaluation provide a strong database for the mixture components.
- Since data are available for either type of methods, we used both approaches to derive Faroe Islands mixture RfDs.

Table 1 - Fish Intake Summary

Source	Media (g)	MeHg in Media (µg/g)	MeHg Daily Intake (µg)	PCB in Media (µg/g)	Daily PCB Intake (µg)	DDT in Media (µg/g)	Daily DDT Intake (µg)
Fish (Mainly Cod)	72	0.075	**5.4**	**----**	**----**	**----**	**----**
Pilot Whale Muscle	12	1.6	**19.2**	0.6	**7.2**	0.3	**3.6**
Pilot Whale Blubber	7	0.17	**1.2**	30	**210**	20	**140**
Total Intake	91		**26**		**217**		**144**

Mixture RfD Based on Component Information

- A first (and key) step - determine the critical effect for each component.
- For example, the critical effect for exposure to MeHg is developmental neurological effect, while for PCB it is an immunotoxicological effect.

Mixture RfD Based on Component Information II

- Enough information exists for the mixture components (IRIS). One method can be used to achieve this goal is the Hazard Index (HI) analysis.
- A general lack of evidence for toxicological interactions between these components allow us to use the no interaction approach, and assumed a dose additivity.

Mixture RfD Based on Component Information III

- Three mixtures component methods:
  - Relative Potency Factor method
  - Toxicity Equivalence Factor method
  - Hazard Index (HI) method.

Mixture RfD Based on Component Information IV

- The first two methods require extensive mechanistic information showing that all the toxic effects of concern share a common mode of action, and this information currently is not available.
- In contrast, the HI method only requires similarity in target organ. Therefore, we choose to use the HI method in deriving the component mixture RfD.

Hazard Index Method I

- MeHg, PCBs and DDT can cause a variety of toxic effects.
- Most sensitive toxic effects (critical health effects) are developmental neurotoxicity, immunotoxicity, and liver toxicity, respectively.

Hazard Index Method II

- Table 2 provides target organ specific effects and their corresponding RfDs.
- For an endpoint that is not the critical effect for that toxicant, an organ specific RfD was estimated based on U.S. EPA non-cancer risk assessment methodology.

Table 2A - RfD Summary for Neurotoxicity Effects

Chemical	IRIS RfD	NT RfD	NT Effect	NOAEL or LOAEL	UF	
Me-Hg	1E-4	1E-4	Developmental NT effects in human infants	BMD 0.0011	10	
PCB (Aroclor 1254)	2E-5	1E-4	Impaired motor coordination in developing rats	0.13	1000	
DDT	5E-4	N/A	N/A	N/A	N/A	

Table 2B - RfD Summary for Immunotoxicity Effects

Chemical	IRIS RfD	IT RfD	IT Effect	NOAEL	UF
Me-Hg	1E-4	N/A	N/A	N/A	N/A
PCB (Aroclor 1254)	2E-5	2E-5	Decreased antibody response to sheep RBCs	LOAEL = 0.005 mg/kg-day	300
DDT	5E-4	N/A	N/A	N/A	N/A

Table 2C - RfD Summary for Systemic Effects

Chemical	IRIS RfD	Systemic RfD	Systemic Effect	NOAEL	UF
Me-Hg	1E-4	5E-4	Bile duct hyperplasia in male rats	0.05	100
PCB (Aroclor 1254)	2E-5	7E-4	Increased liver wt., hepatic hypertrophy and hyperplasia	LOAEL = 0.2 mg/kg-day	300
DDT	5E-4	5E-4	Liver lesions in a 2-year study	0.05 mg/kg-day	100

Hazard Index Comparison

- The HIs are calculated based on EPA mixture guideline (U.S. EPA 1986, 1999) by using the target organ specific RfDs as the reference risk values.
- The results are summarized in Table 3. The calculation equation is:

$$HI = \Sigma(E_i / RfD_i)$$

Where:

E_i is the media contaminant level (mg/kg).

RfD_i is the target organ specific RfD.

Table 3 – Hazard Index Summary

Source	NT HI	IT HI	Liver HI	Mixture RfD (mg/kg-day)
Fish (mainly cod)	**50**	**---**	10	**1E-4** Based on NT
Pilot whale muscle	264	**360**	56	**2E-5** Based on IT
Pilot whale blubber	2111	**10,500**	582	**2E-5** Based on IT
Total intake	2426	**10,860**	648	**2E-5** Based on IT

Mixture RfD Based on Component Information V

- The mixture RfD was estimated by using the following equation (Hertzberg, 1988)

$$RfD_m = TC / \Sigma(E_i / RfD_i)$$

Where:

RfD_m is the mixture RfD.

TC is a total contaminant load in the media (mg/kg)

$TC = \Sigma E_i$

Mixture RfD Based on Component Information VI

- The mixture RfD for consuming whale muscle and blubber would be 2 E-5 mg/kg-day; the mixture RfD for consuming fish would be 1E-4 mg/kg-day.
- For average daily seafood consumption, the mixture RfD would be 2 E-5 mg/kg-day.

Mixture RfD Based on Whole Mixture in Faroe Islands I

- The HI indicates that critical effect is immunotoxicity.
- Data on neurobehavioral effects can be used for a mixtures RfD, but this will likely underestimate risk.
- A mixture RfD is dependent on the composition of the mixture.

Mixture RfD Based on Whole Mixture in Faroe Islands I

- For FI: an average daily intake of 72 g fish, 12 g of whale muscle, and 7 g of whale blubber results in an daily intake of 25.8 µg of MeHg, 217.2 µg of PCB and 143.6 µg of DDT.
- We assumed that this average seafood consumption is constant and the resulting mixtures exposure ratio is also.
- Because mercury exposure in the FI has been extensively studied, we used it as a biomarker in our mixture RfD estimation.

Mixture RfD Based on Whole Mixture in Faroe Islands III

- In 2000, NAS published a document on toxicological effects of MeHg.
- The critical effect was identified as the changes occurred in the Boston Naming Test scores.
- A benchmark dose (BMD) modeling analysis (a 5% extra response) resulted in a BMD of 85 ppb and a BMDL (BMD_{low}) of 58 ppb Hg in cord-blood.

Mixture RfD Based on Whole Mixture in Faroe Islands IV

- Based on the NAS, EPA estimated that the BMDL would be 1.08 µg MeHg/kg by assuming the same Hg concentration occurred in the maternal blood.
- If we assume that the observed responses were also the result of the exposure to PCBs and DDT, then methyl mercury is an ideal mixtures exposure & effect biomarker.

Mixture RfD Based on Whole Mixture in Faroe Islands V

- If the mixture composition is kept constant, the 1.08 µg Me-Hg/kg-day BMDL would correspond to 9.1 µg PCB/kg-day as well as 6.0 µg DDT/kg-day.
- The total mixture exposure would be 1.08 + 9.1 + 6.0 ~ 16 µg mixture/kg-day, the BMDL for intake of the mixture.

Mixture RfD Based on Whole Mixture in Faroe Islands VI

- The mixture RfD can be estimated using an UF of 3 for toxicokinetic variability and 3 for lack of information on immunological responses in FI population. The resulted mixture RfD would be:
- RfD = 16 (mg/kg-day) / 10 = 1.6 µg/kg-day
  = 2 E-3 mg of mixture/kg-day.

Mixture RfD Based on Whole Mixture in Faroe Islands VII

- This value is likely to be too high, because immunotoxicity is not considered in its determination.
- This mixture RfD **must be** limited to neurobehavioral effects (which may not be the critical effects from the mixture exposure
- May underestimate the true risk encountered

Summary – Key Points

- FI cohort a unique data set
- Mixtures RfD can be constructed for fish consumption
- RfD_m based on component data represents a more conservative estimate
- Immunotoxicity effects may be more critical than the neurobehavioral effects