Dioxin-like Activity and Embryotoxic Effects of Unsubstituted and Alkylated Dibenzothiophenes, Benzothiophenes, and Thiophenes

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INTRODUCTION

- Sulfur is the third most abundant element in petroleum constituting 0.05 - 7.5% (w/w) depending on the source of the petroleum.
- Sulfur can be incorporated into polycyclic aromatic hydrocarbons (PAHs) to form polycyclic aromatic sulfur heterocycles (PASHs) such as dibenzothiophene (DBTP), benzothiophene (BTP), and their alkylated homologs.
- DBTP, BTP, as well as other thiophenes (TP), and their alkylated homologs, are predominant forms of organosulfur identified in conventional crude oil and synthetic fuels derived from coal, oil shale, and oil sands.
- Concentrations of total alkylated PAHs are as great as 216,000 µg/g, dw in sediments collected from Athabasca Basin, and DBTPs are the predominant constituents.
- DBTP, BTP, TP, and their alkylated homologs are bioaccumulated and biomagnified in the food chain. However, their toxicity is not fully understood.

OBJECTIVE

- Compare effects of unsubstituted and alkylated DBTPs, BTPs, and TPs on the aryl hydrocarbon receptor (AhR) signaling pathway in vitro by use of the H4IIE-luc assay, and in vivo by use of embryos of fathead minnows.

CHEMICALS

![Chemical structures](image)

**Figure 1.** Nomenclature and structures of chemicals used in this study. A) Dibenzothiophene (DBTP), B) 4-Methyl-Dibenzothiophene (4-Me-DBTP), C) 4,6-Dimethyl-Dibenzothiophene (4,6-diMe-DBTP), D) 1-Benzothiophene (BTP), E) 3-Methyl-Benzothiophene (3-Me-BTP), F) Thiophene (TP), and G) 3-Methyl Thiophene (3-Me-TP).

METHODOLOGY

- H4IIE-luc Assay
  - Cells exposed to chemicals by replacing the culture media with media containing the desired concentration of the chemical of interest.
  - Cells exposed to 0.01, 0.1, 1.0, or 10 mg/L of chemical of interest.
  - Luciferase activity measured as an indicator of activation of the AhR.
  - Antagonistic effects assessed by co-exposing cells to the chemical of interest and 1.85 pM of TCDD because this was the EC50 of TCDD.

Fathead Minnow Embryotoxic Assay

- Eggs were collected within 1 h post-fertilization.
- 10-15 embryos were placed into each well of a 6-well plate.
  - 4 mL solution/well - 50% of volume changed daily.
  - Exposure solutions were at 10 mg/L of chemical of interest.
  - Exposures terminated after 96 h of exposure.
  - Percent of survival, malformation of spine, and pericardial edema quantified.

RESULTS - 1

Antagonistic Effects of DBTPs, BTPs, and TPs on AhR activation

- Co-exposure of H4IIE-luc cells to DBTP, 4-Me-DBTP, and 4,6-diMe-DBTP with 1.85 pM of TCDD resulted in significant lesser activity of luciferase.
- Co-exposure of H4IIE-luc cells to BTP or 4-Me-BTP and 1.85 pM of TCDD resulted in similar effects as DBTPs but the magnitude of effect was lesser, and effects occurred only at the greatest concentration of either chemical.
- Co-exposure of H4IIE-luc cells to TP or 4-Me-TP and 1.85 pM of TCDD did not affect activity of luciferase (data not shown).

RESULTS - 2

Embryotoxic Effects of DBTPs, BTPs, and TPs

- Exposure to 10 mg/L of DBTP, 4-Me-DBTP, or 4,6-diMe-DBTP resulted in significantly lesser survival and greater incidences of malformation of the spine.
- Exposure to 10 mg/L of BTP or 4-Me-BTP did not significantly affect survival but incidences of malformation of the spine were significantly greater.
- Exposure to 10 mg/L of TP and 4-Me-TP did not significantly affect survival or development

CONCLUSIONS

- Neither substituted or unsubstituted DBTPs, BTPs, or TP are agonists of the AhR.
- Substituted and unsubstituted DBTPs or BTPs are antagonists of the AhR.
- DBTPs but not BTPs or TP affect survival of embryos of fathead minnows.
- DBTPs or BTPs, but not TPs, affect development of embryos of fathead minnows.
- Potency of antagonism of the AhR and effects on survival and development of embryo is related to chemical structure.
- Order of potency is (4,6-diMe > 4-Me-DBTP > DBTP) > (3-Me-BTP > BTP)

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