

Case Study on the Use of New Approach Methodologies and Adverse Outcome Pathways for Chemical Hazard Evaluation

Learning objectives:

- Gain familiarity with how to navigate the AOP-Wiki to find information that addresses the relevance of data from “new approach methods” to address a toxicological question.

Instructions:

1. Read through the scenario and charge.
2. Work through the series of questions sequentially, using the public resource provided in the links.

Scenario^a:

Non-targeted analysis of South Carolina drinking water samples identified several unknown peaks in the water supply from a particular community. Follow up investigation via suspect screening identified one of the compounds detected in both the source water and drinking water to be **4-methylaniline**. The mayor has asked state public health officials to advise as to potential health risks and local environmental advocates have raised concerns about effects to resident fish and wildlife. The governor has requested a report from state health and environmental officials by the end of the business week.

Charge: Use the provided information from US EPA’s CompTox Chemicals Dashboard, and the AOP-Wiki to address the following questions:

^a The scenario described is fictional and intended for training purposes. Any resemblance to real events is unintentional.

Part 1. Bioactivity Information in the CompTox Chemicals Dashboard

The following information on the bioactivity of 4-methylaniline (also known as 4-methylbenzenamine) was obtained from US EPA's CompTox Chemicals Dashboard (CCD). Click the [link to view on-line](#).



- 4-methylaniline exhibits specific bioactivity at concentrations well below those that lead to more systemic (non-specific) toxicity.
 - Bioactivity at concentrations near or above the “Cytotoxicity Lower Bound” is generally non-specific and reflective of systemic damage.
 - 4-methylaniline has several specific biological activities at concentrations over 10 times lower than the Cytotoxicity Lower Bound.
- The most sensitive bioactivity is in the Ccte_Simmons_AUR_TPO assay (AC50 = 0.083 μ M), followed by the Ccte_Simmons_GUA_TPO (AC50 = 4.9 μ M) assay. Both assays measure effects on the catalytic activity of thyroid peroxidase (TPO).
 - On the bioactivity summary plot, these are the two points farthest left along the X-axis (AC50 in μ M).
 - This can also be viewed in the “Bioactivity Summary Grid” immediately below the TOXCAST bioactivity summary plot. The Grid can be sorted by AC50 (lowest to highest) to identify the most sensitive assay responses.

Part 2. Inter-operability between CCD and AOP-Wiki

3. **Are there any “OECD Endorsed AOPs” associated with this bioactivity (thyroid peroxidase [TPO] inhibition)?**
 - a. Hint –On the [4-methylaniline Bioactivity page](#) scroll down to the “Bioactivity Summary Grid”, sort the table lowest to highest by AC50 or log AC50, examine the AOP column (column 6) and Event column (column 7).

4. **What are the titles of these AOPs?**
 - a. Hint - The AOP Column (column 6 of the Bioactivity Grid) indicates there are two endorsed AOPs associated with the TPO inhibition bioactivity, AOPs [159](#) and [42](#).
 - b. You can click on either AOP number to be redirected to the corresponding AOP page in the AOP-Wiki.

5. **What is the domain of applicability (sex, life stage, taxa) of these OECD Endorsed AOPs?**
 - a. Hint – on the Table of Contents on the left side of the AOP page, click on “Domain of Applicability” found under “Overall Assessment of the AOP”

6. **Based on the OECD Endorsed AOPs, what is a potential hazard to humans exposed to 4-methylaniline? What is a potential hazard to aquatic wildlife?**
 - a. Hint – AOP titles generally take the form “[Molecular initiating event] lead to [Adverse Outcome] via [A distinctive Key Event]”.

7. **Are there other AOPs, under development, that are linked to TPO inhibition as a Key Event? If so, what are some of the other hazards/adverse outcomes that might be associated?**
 - a. Hint - The “Event” Column (column 7 of the Bioactivity Grid) identifies a Key Event in the AOP-Wiki that corresponds to the TPO inhibition bioactivity.
 - b. You can click on the Event 279 link to be redirected directly to the Event 279 page in the AOP-Wiki.

- c. On Event Page 279 navigate down to “AOPs Including this Key Event”
- d. What are some of the other adverse outcomes suggested by the titles?

8. Besides the ToxCast assays (assays from part 1 above), what are some of the methods that could be used to measure TPO activity?

- a. Hint – on Event Page 279, navigate down to “How it is Measured or Detected”

Part 3. Searching and Navigating the AOP-Wiki

This section will guide you through how to find similar information directly in the AOP-Wiki, without using the links found in the bioactivity tab of the CCD.

9. Are there any other OECD Endorsed AOPs relevant to TPO (thyroid peroxidase; thyroperoxidase) that were not listed in the CCD?

- a. Go to aopwiki.org
- b. Under “View Content” click on “AOPs”
- c. In the “Search AOPs...” field, type a search term (e.g., TPO; thyroperoxidase; thyroid peroxidase).
- d. Click the drop-down menu in the “OECD Status” column and select “WPHA/WNT Endorsed”

10. How much confidence did the developer(s) of AOP 42 have in the relationship between TPO inhibition and decreased cognitive function?

- a. In the “Find By ID” field, enter “42” and click on “Find By ID” – note, the Find by ID will not work if you just hit “Enter”.
- b. On [AOP page 42](#), scroll or navigate down to “Relationships Between Two Key Events”.
- c. Examine the calls in the “Evidence” column for each Relationship.
- d. For more detail, navigate to the “Overall Assessment of the AOP” (but in the interest of time, skip that for today).

11. Do you find the evidence linking thyroid peroxidase inhibition to decreased serum T4 ([Relationship 366](#)) compelling?

- a. From AOP Page 42, “Relationships Between Two Key Events” Table, click the link for [Thyroperoxidase, Inhibition leads to T4 in Serum, Decreased](#).
- b. Note the URL at the top of the page is now for a Relationship.
- c. Scroll or navigate to the section titled “Evidence Supporting this KER” – for today you may want to just quickly skim.

12. Based on a AOP search using the term “thyroperoxidase” you also identify an AOP linking thyroperoxidase inhibition to altered visual function via altered retinal layer structure ([AOP 363](#)). This AOP is not WPHA/WNT Endorsed, but is it sufficiently developed to support identification of altered visual function as a potential hazard?

- a. Navigate to AOP page 363 by either clicking the link to the title from the AOP search page or conducting a “Find by ID” search for 363.
- b. Scroll down the AOP page – how developed does it look?
- c. From the “Relationships Between Two Key Events” table, click on one of the relationships. How well developed is the relationship page?
- d. Use “Back” on your Browser or click on the AOP 363 title in the “AOPs Referencing Relationship” table on the Relationship page to return to AOP page 363.
- e. Scan another relationship page or two using the same approach (repeating 12c, 12d).

13. Are the potential effects on retinal layer development relevant to humans?

- a. On AOP page 363, scroll or navigate down to Domain of Applicability.
- b. You may also want to navigate to one or more relationships (e.g., [Relationship 2373 linking Decreased, Triiodothyronine \(T3\) to altered retinal layer structure](#)), Evidence Supporting the Domain of Applicability. What is the taxonomic applicability of the relationship?

14. Continue to explore on your own...

Based on what you found using the CompTox Chemicals Dashboard and AOP-Wiki, what are some of the potential hazards to humans and/or aquatic wild-life to mention in your preliminary report to the governor and what could you cite for support?
