Linking an In Vivo Pregnancy Loss Model to an In Vitro Placental Cell System to Explore the Mode of Action of Disinfection Byproducts

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LTG 1 Poster 04

Science Questions

What is the mode of action of toxicant-induced pregnancy loss in rodent models?

Do disinfection byproducts (DBPs) alter human placental function and is this related to human pregnancy loss following exposure to DBPs?

What is the cumulative effect of DBPs and other water contaminants on pregnancy maintenance and placental function?

Research Goals

- To understand the plausibility and biological basis for human pregnancy loss associated with drinking water containing disinfection byproducts (DBPs).
- To determine the mode of action of trihalomethane (THM)- and bromodichloromethane (BDCM)-induced pregnancy loss in a rodent model, and to determine if other DBPs and DBP mixtures also cause this effect.
- To investigate the effects of BDCM and DBP mixtures on human placental trophoblast function in vitro.

Chemicals causing pregnancy loss in F344 rats

Pesticides	Solvents
Atrazine & metabolites	Carbon tetrachloride
Amitraz	Trichloroethylene
Thiram	Tetrachloroethylene

Disinfection byproducts
Bromodichloromethane, Bromoform
Haloacetic acids in mixture

DBP Mixtures in Rodent Model
When administered by gavage, a mixture of four trihalomethanes and a mixture of five haloacetic acids each caused pregnancy loss in F344 rats. When combined, both chemical classes contributed to the potency of the nine-DBP mixture. When administered by drinking water, a chlorinated concentrate (136x) did not disrupt pregnancy in F344 or Sprague-Dawley rats.

Findings and Conclusions

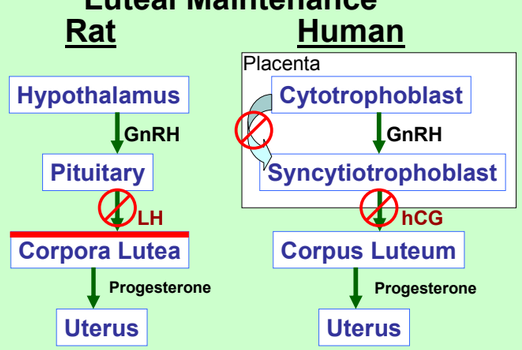
LH-mediated mode of action for BDCM-induced pregnancy loss in F344 rats
- Effect requires exposure during the LH-dependent period of pregnancy.
- Decreased serum levels of LH (and progesterone) in rats with pregnancy loss.
- Treatment with hCG, an LH agonist, rescues pregnancies.
- Corpora lutea cultured in BDCM are less responsive to hCG stimulation in vitro, suggesting altered LH responsiveness.

Strain Differences
The F344 strain is particularly sensitive to bromodichloromethane-induced pregnancy loss, the Long-Evans stock is moderately sensitive, and the Sprague Dawley stock appears to be insensitive. Lower baseline levels of LH in F344 rats are associated with susceptibility to this LH-mediated effect.

In vitro Human Trophoblast Model
Bromodichloromethane inhibited trophoblast differentiation and hCG secretion in vitro at concentrations within the range of reported human blood levels. A whole mixture of DBPs (136x) as well as a defined mixture of regulated DBPs also inhibited differentiation and hCG secretion.

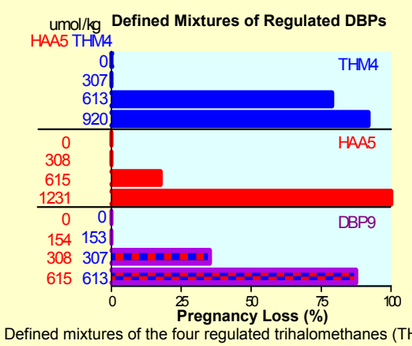
Methods/Approach

Luteal Maintenance



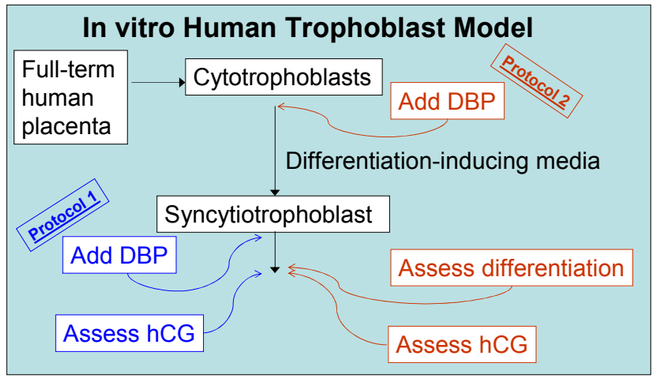
In rats, during the LH-dependent period of pregnancy (days 7-10), GnRH stimulates secretion of luteinizing hormone (LH), which in turn stimulates luteal secretion of progesterone. BDCM has been shown to disrupt LH secretion and LH responsiveness. In humans, chorionic gonadotropin (hCG, a hormone similar to LH) from the placenta maintains luteal progesterone secretion. GnRH from cytotrophoblast cells putatively modulate hCG secretion. BDCM has been shown to inhibit hCG secretion as well as differentiation from cytotrophoblasts to multinucleated syncytiotrophoblast.

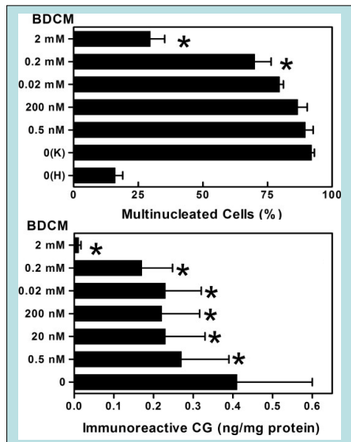
In vivo Rodent Model

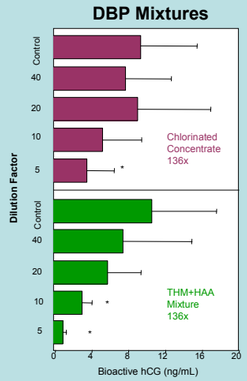


Defined mixtures of the four regulated trihalomethanes (THM4), the five regulated haloacetic acids (HAA5), and THM4+HAA5 (DBP9) each caused pregnancy loss (i.e., full-litter resorption) at ≥613 umol/kg. Both THMs and HAAs contributed to the potency of DBP9.

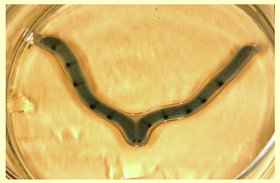
In vitro Human Trophoblast Model



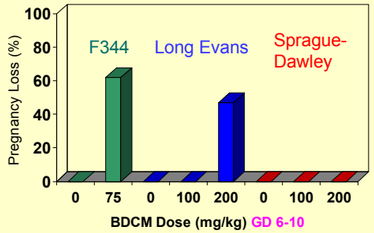




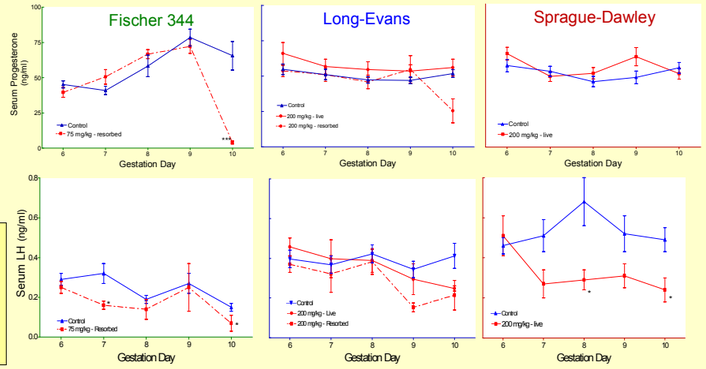
BDCM (top left) and DBP mixtures inhibited differentiation of human cytotrophoblasts in vitro. The lowest effective concentration of BDCM was within the range of human reported blood levels. BDCM (bottom left) and DBP mixtures (right) reduced hCG secretion of trophoblasts in vitro. Two mixtures were evaluated: chlorinated concentrate (136x compared to tap water) and a matching mixture with only regulated THMs and HAAs.



Staining with ammonium sulfide shows resorption sites in a uterus from a trichloroethylene-treated dam with pregnancy loss (i.e., full-litter resorption).



Strain Comparisons of BDCM-Induced Pregnancy Loss. F344 is the most sensitive strain tested. Long Evans rats are moderately sensitive; Sprague-Dawley rats appear resistant. All strains show reductions in serum LH; however, higher baseline LH levels in less sensitive strains may reduce their susceptibility to DBP-induced pregnancy loss.



Impact and Outcomes

We have demonstrated that mixtures of trihalomethanes (THMs) and haloacetic acids (HAAs) both cause pregnancy loss in a rodent model. HAA-induced pregnancy loss has not been previously reported. It remains unclear whether the HAAs act via the same mode of action as the THMs. However, the finding that HAAs contribute to the potency of THMs in mixture is consistent with dose additivity and suggests a similar mode of action.

Using an in vitro model, we have demonstrated that DBP mixtures directly perturb human placental function. Defined mixtures of regulated DBPs (THMs and HAAs) had similar effects as a chlorinated concentrate "whole" mixture, suggesting that regulating the THMs and HAAs may provide adequate protection from DBP-induced pregnancy loss.

Taken together, the rodent model in vivo and the human placental model in vitro suggest that there is a biological basis for DBPs to induce human pregnancy loss that may be mediated by changes in hormone production and hormone-induced biological functions required to maintain pregnancy.

The combination of the whole-mixture results, defined-mixture results, and single compound effects provides an approach toward understanding how drinking water exposures may affect human placental function. Understanding the mechanism and exposure-dose-effect relationships will better inform regulators and risk assessors of the protection that current regulation provides to unborn children.

Future Directions

- Investigate the mechanisms for DBP disruption of placental function.
- Investigate the cumulative actions of HAAs with each other and with THMs for their ability to cause pregnancy loss in rats.
- Explore strain differences in susceptibility to toxicant-induced pregnancy loss. In conjunction with mode-of-action information, strain differences may provide insights into high-risk human populations.

Researchers Involved

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