Analysis of Eight Oil Spill Dispersants For Cell Toxicity and Estrogenic Activity

Richard Judson
EPA Center for Computational Toxicology and Exposure

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Deepwater Horizon

Oil Exploration Platform Explodes April 20, 2010

- Estimated 4.9 million barrels of South Louisiana Crude released

1.8 million gallons of dispersant used

- 1072K surface; 771K subsea
- Corexit 9500A (9527 early in spill)

EPA Administrator called for a less toxic alternative

- Verification of toxicity information on NCP Product Schedule
- EPA involvement in assessments of dispersant toxicity

EPA Oil Dispersant Oversight

- Clean Water Act & Oil Pollution Act (1990)
- EPA Office of Emergency Management
  - National Oil and Hazardous Substances Pollution Contingency Plan (https://www.epa.gov/emergency-response/ncp-product-schedule-products-available-use-oil-spills)
  - NCP (National Contingency Plan) Product Schedule Categories
    - Dispersants
    - Surface washing agents
    - Bioremedial agents
    - Misc (sorbents, solidifiers, etc.)
- Inclusion on schedule does not authorize use
- Product information (toxicity, effectiveness) must be supplied by manufacturer (40 Code of Regulations, Part 300, Subpart J, Appendix C)

EPA United States Environmental Protection Agency

What is a dispersant?

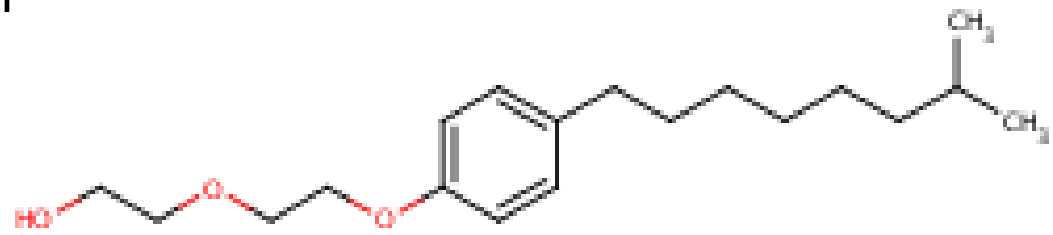
- Complex mixture
- Proprietary / Confidential Business Information
- Hydrocarbon component
  - Breaks up clumps of oil
  - Like kerosene
- Detergent / surfactant component
  - Solubilizes oil components into water
- Water
- Colorants
- Stabilizing agents

What Distinguishes One Dispersant from Another?

- Efficacy
  - How well it disperses oil
  - EPA does not regulate on this
- Toxicity
  - How much of the dispersant is required to be toxic
    - Units are volume dispersant / volume of water in ppm
    - Menidia beryllina – Silverside minnow
    - Mysidopsis bahia – Mysid / opossum shrimp
  - Special concern for estrogenic activity for the Deepwater Horizon spill

Why Worry About Estrogen Activity?

- It was believed that some of the dispersants contained nonylphenol ethoxylate or related compounds
  - These degrade in the environment to compounds that mimic natural estrogen
  - Recall that the composition of the dispersants is proprietary
- Estrogen is a natural hormone that is required for sexual development and maturation, maintaining bone health and many other processes
- However, exposure at the wrong time and wrong level can lead to significant health issues
  - Sex change in fish and amphibians (intersex)
  - Infertility
  - Reproductive tract cancers (e.g. from DES)
- Injecting estrogens into Gulf Coast breeding grounds could cause crash in populations of fish and shri



EPA Toxicity Studies

- Rerun standard toxicity studies to confirm results submitted by companies
  - Menidia beryllina – Silverside minnow
  - Mysidopsis bahia – Mysid / opossum shrimp
  - No significant differences seen from industry-supplied values
- Do these studies with oil + dispersant to see if there are synergetic effects
  - No significant differences seen vs. no oil
- Run studies in human cells to determine:
  - Level of dispersants required to kill human cells
  - Presence of estrogen-like activity
  - Presence of androgen (testosterone)-like activity, AR [none seen]

Dispersants Considered for Use

Sample Name	Volume Received	Comments	Date Received	Manufacturer/ Source
Corexit 9500	1 L	hazy yellow	11-May-10	Nalco
JD 2000	10 ml	clear yellow	27-May-10	Ethox Chemicals, LLC
DISPERSIT SPC 1000	10 ml	clear amber	27-May-10	Polychem
Sea Brat #4	10 ml	hazy yellow	27-May-10	Alabaster Corp
Nokomis 3-AA	10 ml	clear light color	27-May-10	MAR-LEN Supply inc.
Nokomis 3-F4	10 ml	clear light color	27-May-10	MAR-LEN Supply inc.
ZI-400	25 ml	clear yellow	29-May-10	ZI Chemical
SAF-RON GOLD	500 ml	silver iridescent	4-June-10	Sustainable Environmental Technologies, Inc.

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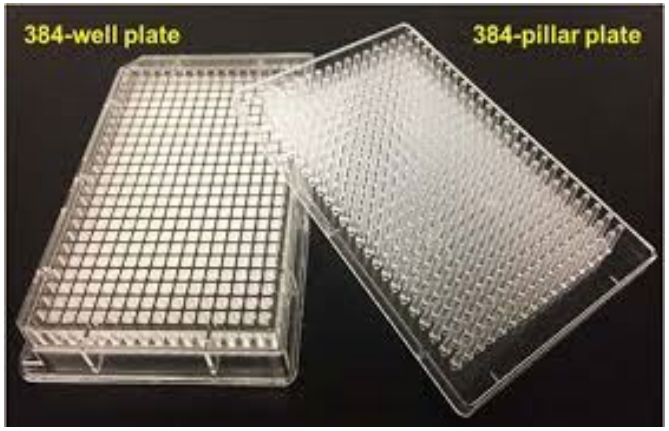
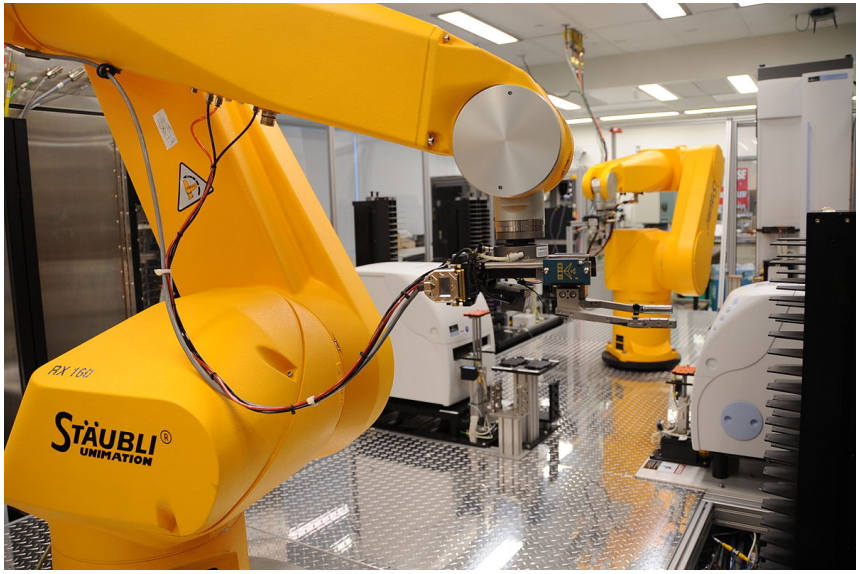
A Little Molecular Biology

- Estrogen and related compounds act by binding to a protein called the Estrogen Receptor (ER)
- ER with a bound estrogenic compound causes specific genes to be up-regulated
- These genes in turn cause specific types of cells to divide and multiply
  - Example is growth and maturation of the uterus during puberty
- In specific types of human cells, we can measure gene changes and cell proliferation in response to estrogenic compounds
  - "In vitro assays"

In Vitro Assay Technology

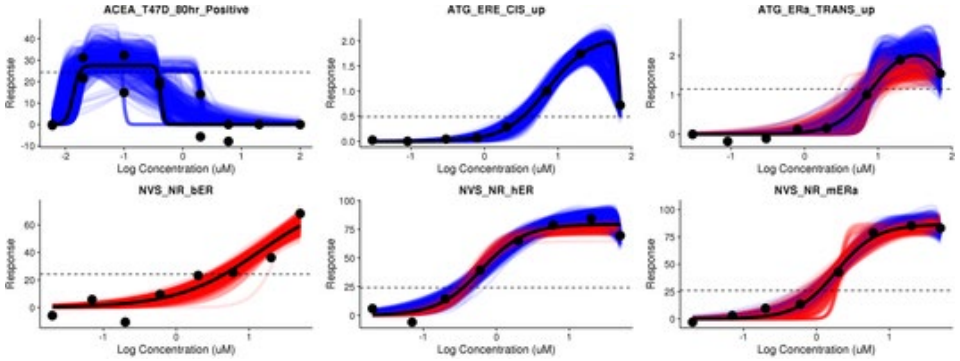


Cells and chemicals added to plates …



Using big and little robots (and people) …

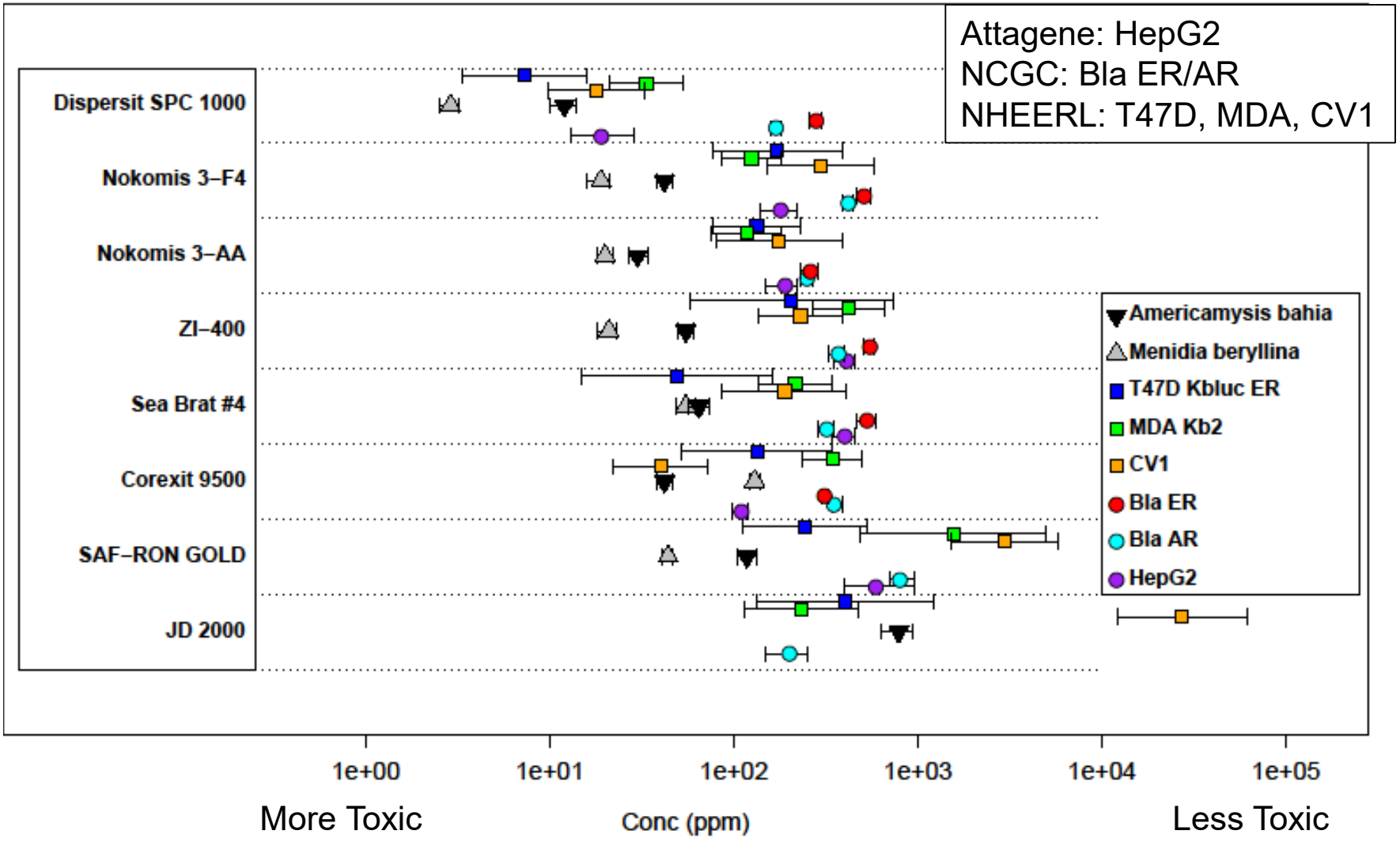
… resulting in response vs. concentration data



In Vitro Assay Technologies Used

- Competitive binding (Novascreen)
  - Cell-free
  - Dispersants seem to have denatured proteins, given non-specific results
  - Will not discuss further
- ER/AR reporter-gene assays (NCGC)
  - Agonist and antagonist mode
  - Cytotoxicity (cell killing)
- Collection of 81 nuclear-receptor-related assays (Attagene)
  - Includes ER, AR, thyroid receptor
  - Cytotoxicity

Cytotoxicity Results

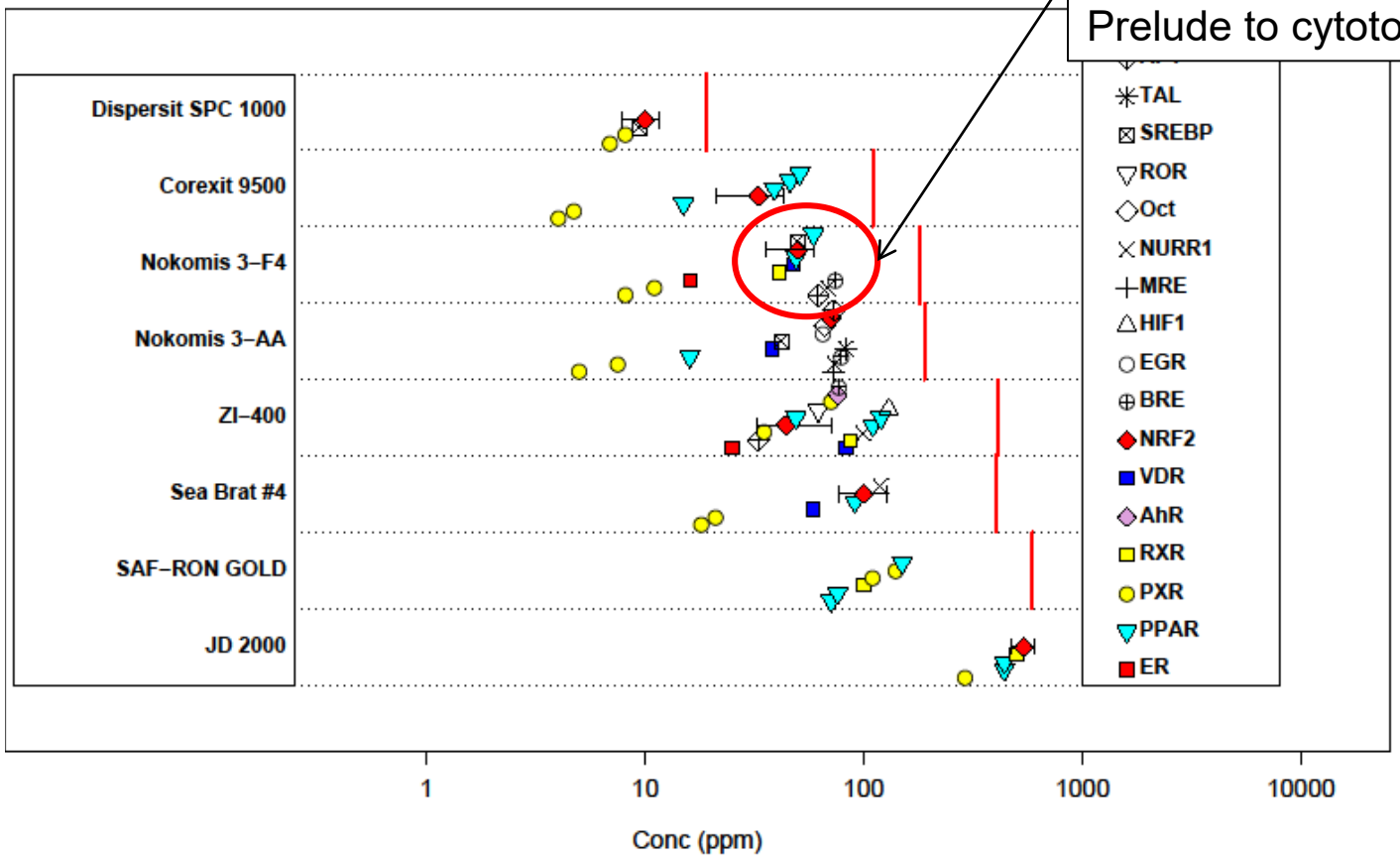


Endocrine Assay Results

- No AR activity
- Weak ER activity seen for 2 dispersants in on ER assay
  - Nokomis 3-F4
  - Sea Brat 4
- Both of these (probably) contain nonylphenol ethoxylates
  - CEO of Alabaster (Sea Brat) says Sea Brat contains <2% NPE
  - Nokomis web site said they have alternative formulations without NPE, implying standard formulation includes NPE

Other In Vitro Assay Results

Multi-assay pile-up
Indication of generalized cell stress
Prelude to cytotoxicity



Data indicates up-regulation of liver metabolism genes (detoxification)

Dispersant Conclusions

- Weak evidence of ER activity in 2 dispersants
  - Seen in single, perhaps over-sensitive assay (1 of 6)
  - Not of biological significance
  - Consistent with presence of NPE
  - Activity only at concentrations >> seen in Gulf after dilution
- No AR activity
- No ER activity seen in Corexit 9500
- Corexit is in the middle of the pack for cytotoxicity
- EPA recommended continued use of Corexit 9500

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- judson.richard@epa.gov