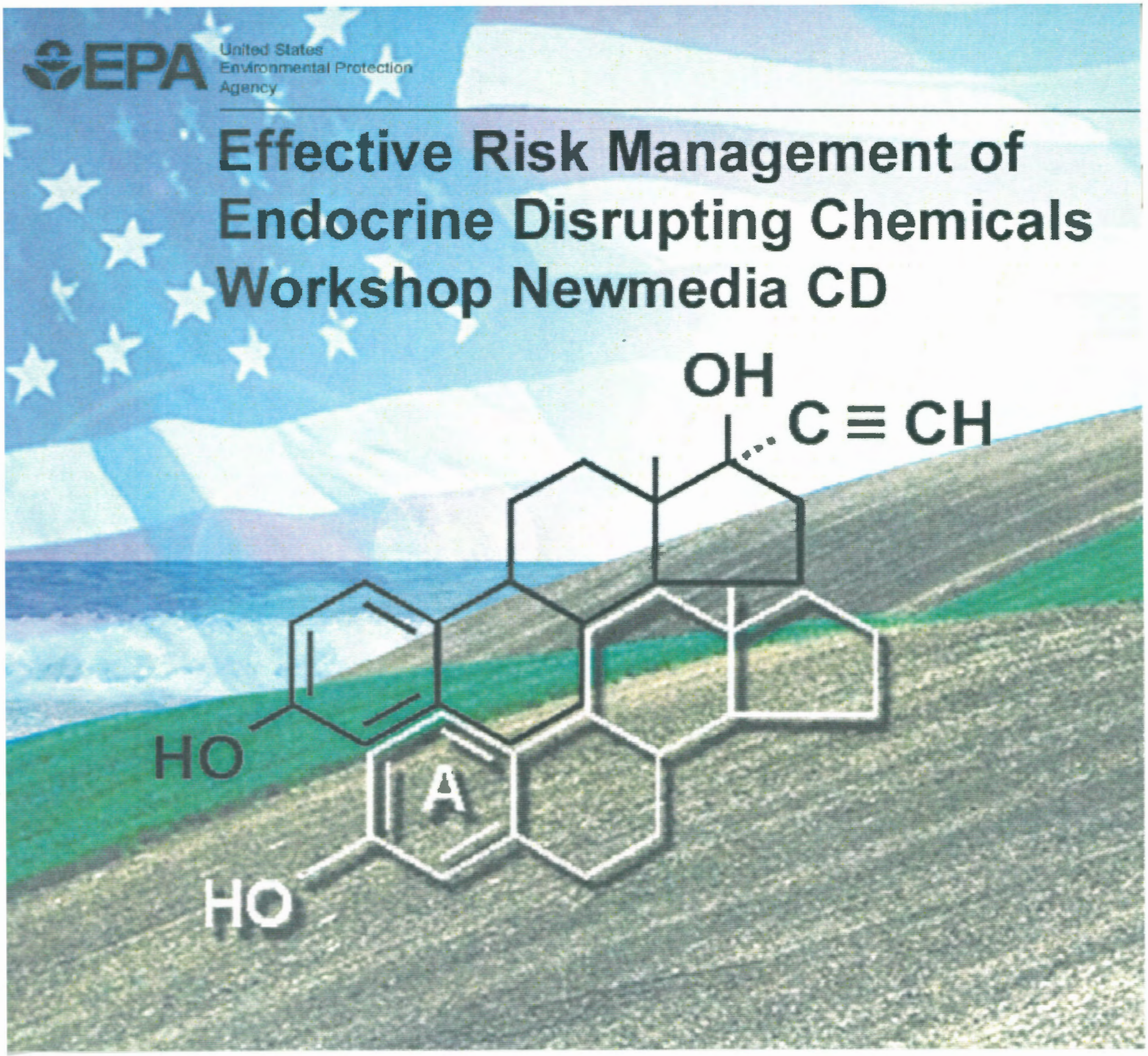

EPA
United States
Environmental Protection
Agency
Effective Risk Management of
Endocrine Disrupting Chemicals
Workshop Newmedia CD
OH
C ≡ CH
HO
A
HO

Effective Risk Management of Endocrine Disrupting Chemicals

Vernon Manor Hotel
Cincinnati, OH
January 29-30, 2002

Final Agenda

✦Tuesday, January 29, 2002✦

7:00-8:20 AM Registration

8:30 AM *Welcome and Logistics*
John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

Risk Management Context
Moderator: Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

8:35 AM *Purpose and Goals of the Workshop*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

8:40 AM *Welcome from the National Risk Management Research Labora*tory
Lee Mulkey, U.S. EPA, National Risk Management Research Laboratory

8:50 AM *Risk Management Research: Improving Environmental Decisions*
Hugh McKinnon, U.S. EPA, National Risk Management Research Laboratory

9:20 AM *European Community Strategy for Endocrine Disruptors: Implementation to Date*
Kathryn Tierney, European Commission

9:50 AM Break

Effects of EDCs on Humans and Wildlife
Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

10:10 AM *Introduction to EDCs and Their Potential Effects on Humans*
Ralph Cooper, U.S. EPA, National Health and Environmental Effects Laboratory

10:50 AM *Overview of Effects and Assessment of Endocrine-Disrupting Chemicals in Wildlife*
Gary Ankley, U.S. EPA, National Health and Environmental Effects Laboratory

11:30 AM *EPA's Endocrine Disruptors Screening Program: Legislation, Implementation, and Research*
Elaine Francis, U.S. EPA, National Center for Environmental Research

12:00 PM Lunch

Exposure Assessment for EDCs
Moderator: John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

1:10 PM *Development of Biological Methods to Characterize Exposure of Wildlife to EDCs*
Greg Toth, U.S. EPA, National Exposure Research Laboratory

1:40 PM *Development of Chemical Methods to Characterize Exposure to EDCs in the Neuse River Basin*
Myriam Medina-Vera, U.S. EPA, National Exposure Research Laboratory

2:10 PM *Monitoring Endocrine Disrupting Compounds in Aquatic Ecosystems in the United States*
Steve Goodbred, U.S. Geological Survey

2:40 PM *Region 5: Ongoing Endocrine Disruptor Efforts*
Lawrence Zintek, U.S. EPA, Region 5

3:10 PM Break

3:30 PM *Residential Indoor Air and Dust Measurements of Phthalates and Other Endocrine Disrupting Compounds*
Ruthann Rudel, Silent Spring Institute

Risk Management Approaches
Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

4:00 PM *EPA's Risk Management Evaluation of EDCs*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

4:40 PM *Using Bioassays to Evaluate the Performance of Risk Management Techniques*
Carolyn Acheson, U.S. EPA, National Risk Management Research Laboratory

5:10 PM Adjourn for the day

✦Wednesday, January 30, 2002✦

8:30 AM *Welcome and Logistics for Day 2*
John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

Drinking Water Treatment
Moderator: James Goodrich, U.S. EPA, National Risk Management Research Laboratory

8:35 AM *Use of Granular Activated Carbon and Powdered Activated Carbon for the Removal of EDCs from Drinking Water: A User's Guide*
John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

9:05 AM *Evaluation of Drinking Water Treatment Technologies for Removal of Endocrine Distrupting Compounds*
Kathleen Schenck, U.S. EPA, National Risk Management Research Laboratory

9:20 AM *Risk Management of Endocrine Disrupting Chemicals (EDCs) in Drinking Water*
Frederick Pontius, Pontius Water Consultants, Inc.

9:50 AM Break

Concentrated Animal Feed Operations
Moderator: Laurel Staley, U.S. EPA, National Risk Management Research Laboratory

10:10 AM *Potential of Confined Animal Feed Operations (CAFOs) to Contribute Estrogens to the Environment*
Steven Hutchins, U.S. EPA, National Risk Management Research Laboratory

10:40 AM *Investigations of Sorption and Transport of Hormones and Animal Pharmaceuticals: Initial Laboratory Results*
Suresh Rao, Purdue University
Linda Lee, Purdue University

11:00 AM *Fate of the Endogenous Hormones 17ß-Estradiol and Testosterone in Composted Poultry Manure and their Sorption and Mobility in Loam Soil and Sand*
Heldur Hakk, USDA, Agricultural Research Service

11:30 AM Lunch

Wastewater Treatment
Moderator: Marc Mills, U.S. EPA, National Risk Management Research Laboratory

12:45 PM *Biological Fate of Estrogenic Compounds Associated with Sewage Treatment*: *A Review*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

1:15 PM *An Engineering Approach to Evaluate Estrogenic EDCs Fate During Wastewater Treatment*
Paul McCauley, U.S. EPA, National Risk Management Research Laboratory

1:30 PM Break

Other EDC Risk Management Challenges
Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

1:45 PM *Endocrine Disruptors from Combustion and Vehicular Emissions: Identification and Source Nomination*
Brian Gullett, U.S. EPA, National Risk Management Research Laboratory

2:00 PM *Natural Recovery of PCB-Contaminated Sediments at the Sangamo-Weston / Twelve Mile Creek / Lake Hartwell Superfund Site*
Richard Brenner, U.S. EPA, National Risk Management Research Laboratory
James Lazorchak, U.S. EPA, National Exposure Research Laboratory

2:45 PM *Program for the Identification and Replacement of Endocrine Disrupting Chemicals*
Douglas Young, U.S. EPA, National Risk Management Research Laboratory

3:00 PM Adjourn Workshop

Updated January 18, 2002

Jim Abbott
OFFICE: 614-424-7781
FAX: 614-424-3667
abbottj@battelle.org
Battelle
505 King Avenue
Columbus, OH 43201 USA

Carolyn Acheson
OFFICE: 513-569-7190
FAX: 513-569-7105
acheson.carolyn@epa.gov
US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Bruce Alleman
OFFICE: 614-424-5715
FAX: 614-424-3667
allemanb@battelle.org
Battelle
505 King Avenue
Columbus, OH 43201 USA

Al Alwan
OFFICE: 312-353-2004
FAX: 312-886-0168
alwan.al@epa.gov
US EPA Region 5
77 W. Jackson Boulevard
Chicago, IL 60604 USA

Gerald T. Ankley
OFFICE: 218-529-5147
FAX: 218-529-5003
ankley.gerald@epa.gov
US EPA
6120 Congdon Boulevard
Duluth, MN 55804 USA

Barry Austern
OFFICE: 513-569-7638
FAX: 513-569-7105
austern.barry@epa.gov
US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Andy Avel
OFFICE: 513-569-7951
FAX: 513-569-7680
avel.andy@epa.gov
US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

John Bankston
OFFICE: 412-778-3315
FAX: 412-778-3545
bankstonjr@sunocochemicals.com
Sunoco, Inc.
200 Neville Road
Neville Island, PA 15225 USA

Joe Bartoszek
OFFICE: 937-285-6357
FAX:
joe.bartoszek@epa.state.oh.us
Ohio EPA
401 E. Fifth Street
Dayton, OH 45402 USA

Thomas L. Baugh
OFFICE: 404-562-8275
FAX: 404-562-8269
baugh.thomasl@epa.gov
US EPA
61 Forsyth Street, SW
Atlanta, GA 30303 USA

Scott Bergreen
OFFICE: 740-380-5288
FAX: 740-385-6490
scott.bergreen@epa.state.oh.us
Ohio EPA
2195 Front Street
Logan, OH 43107 USA

Gwyn Boehringer
OFFICE: 937-445-1463
FAX: 937-445-1461
gwyn.boehringer@ncr.com

NCR
1611 South Main Street, ORGH
Dayton, OH 45479-0001 USA

Rick Booth
OFFICE: 636-936-1554
FAX: 636-936-1535
rbooth@golder.com

Golder Associates, Inc.
1630 Heritage Landing
St. Charles, MO 63303-8492 USA

Dick Brenner
OFFICE: 513-569-7657
FAX: 513-569-7105
brenner.richard@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Elizabeth A. Bruewer
OFFICE:
FAX:
bruewer@bgnet.bgsu.edu

Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Ed Burcham
OFFICE: 513-626-0715
FAX: 513-626-0115
burcham.pe@pg.com

P&G
11450 Grooms Road
Box C13
Cincinnati, OH 45242-1434 USA

Sarah N. Bush
OFFICE:
FAX:
sbush@bgnet.bgsu.edu

Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Greg Buthker
OFFICE: 937-285-6445
FAX:
greg.buthker@epa.state.oh.us

Ohio EPA
401 East Fifth Street
Dayton, OH 45402 USA

Ronald Chambers
OFFICE: 513-352-2922
FAX: 513-352-2915
ronald.chambers@rcc.org

Cincinnati Health Department
1525 Elm Street
Cincinnati, OH 45210 USA

Lina Chang
OFFICE: 513-569-7442
FAX: 513-569-7609
chang.lina@epa.gov

US EPA
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Russ Christenson
OFFICE: 913-433-5225
FAX: 913-433-5125
russ.christenson.b@bayer.com

Bayer Corporation
17745 S. Metcalf
Stilwell, KS 66085-9104 USA

U. Eric Chukwu
OFFICE: 703-784-4472
FAX: 703-784-3432
chukwueu@mcsc.usmc.mil

MARCORSYSCOM
Program Support Directorate
2033 Barnett Avenue, Suite 315
Quantico, VA 22134-5010 USA

Michael Chung
OFFICE: 201-398-4314
FAX: 201-797-4399
mchung@pirnie.com

Malcolm Pirnie, Inc.
17-17 Route 208 N.
Fair Lawn, NJ 07410 USA

John Cicmanec
OFFICE: 513-569-7481
FAX: 513-569-7585
cicmanec.john@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
MS G75
Cincinnati, OH 45268 USA

Linda M. Clark
OFFICE: 215-299-6133
FAX: 215-299-6947
linda_clark@fmc.com

FMC Corporation
1735 Market Street
Philadelphia, PA 19103 USA

Robert M. Clark
OFFICE: 513-569-7201
FAX: 513-569-7658
clark.robertm@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Pat Cline
OFFICE: 352-336-5600
FAX: 352-336-6603
pcline@golder.com

Golder Associates
6241 N.W. 23rd Street
Suite 500
Gainesville, FL 32653 USA

Joan Colson
OFFICE: 513-569-7501
FAX: 513-569-7585
colson.joan@epa.gov

US EPA ORD
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Betsy Cooper
OFFICE: 206-263-3728
FAX: 206-684-1741
betsy.cooper@metrokc.gov

King County DNR
Wastewater Treatment Division
201 S. Jackson Street
Seattle, WA 98104 USA

Ralph Cooper
OFFICE: 919-541-4084
FAX: 919-541-5138
cooper.ralph@epa.gov

US EPA NHEERL
MD-72
Research Triangle Park, NC 27711 USA

Easter A. Coppedge
OFFICE: 919-541-7863
FAX: 919-541-3527
coppedge.easter@epa.gov

US EPA
MD-44
Research Triangle Park, NC 27711 USA

Christie Croften
OFFICE:
FAX:
ccrofte@bgnet.bgsu.edu

Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Wendy Davis-Hoover
OFFICE: 513-569-7206
FAX:
davis-hoover.wendy@epa.gov

US EPA NRMRL
5995 Center Hill Avenue
Cincinnati, OH 45224 USA

Armah de la Cruz OFFICE: 513-569-7224 FAX: 513-569-7170 delacruz.armah@epa.gov	**US EPA NERL / MCEARD / MERB** 26 W. Martin Luther King Drive Cincinnati, OH 45268-1314 USA
Shawn Dempsey OFFICE: FAX: shawnd@bgnet.bgsu.edu	**Bowling Green State University** Environmental Health Program 223 Health Center Bowling Green, OH 43403 USA
Anne Donlin OFFICE: 513-569-4801 FAX: 513-569-4800 adonlin@queencity.com	**SAIC** 2260 Park Avenue Suite 402 Cincinnati, OH 45206 USA
Mike Elovitz OFFICE: 513-569-7642 FAX: elovitz.michael@epa.gov	**US EPA NRMRL** 26 W. Martin Luther King Drive Cincinnati, OH 45268 USA
Carl Enfield OFFICE: 513-569-7489 FAX: enfield.carl@epa.gov	**US EPA** 26 W. Martin Luther King Drive Cincinnati, OH 45268 USA
Gloria Ferrell OFFICE: 919-571-4057 FAX: 919-571-4041 gferrell@usgs.gov	**US Geological Survey** 3916 Sunset Ridge Road Raleigh, NC 27607 USA
Dean Finney OFFICE: 423-378-4120 FAX: 423-378-4120 dfinney@chartertn.net	**RIVENDELL Consultants** 1233 Morningside Circle Kingsport, TN 37664 USA
Nelson Fok OFFICE: 780-413-7936 FAX: 780-482-5383 nfok@cha.ab.ca	**Capital Health, Alberta** 10216-124 Street 300 Edmonton, Alberta T5N4A3 CANADA
Eric Foote OFFICE: 614-424-7939 FAX: 614-424-3667	**Battelle** 505 King Avenue Columbus, OH 43201 USA
Elaine Francis OFFICE: 202-564-6789 FAX: 202-565-2444 francis.elaine@epa.gov	**US EPA NCER** 1200 Pennsylvania Ave., N.W. 8701R Washington, DC 20460 USA
Patty Gallagher OFFICE: 513-569-7579 FAX: 513-569-7757 gallagher.patricia@epa.gov	**US EPA NERL** 26 W. Martin Luther King Drive Cincinnati, OH 45268 USA

Achal Garg
OFFICE: 513-557-7034
FAX: 513-557-7050
achal.garg@rcc.org

City of Cincinnati
1600 Gest Street
Cincinnati, OH 45204 USA

Dennis B. George
OFFICE: 931-372-3507
FAX: 931-372-6346
dgeorge@tntech.edu

Tennessee Technological University
1020 Stadium Drive
P.O. Box 5033
Cookeville, TN 38505 USA

Emma Lou George
OFFICE: 513-569-7578
FAX: 513-569-7585
george.emmalou@epa.gov

US EPA ORD / NRMRL / TTSD / TTB
26 W. Martin Luther King Drive
Cincinnati, OH 45268-0001 USA

Susan Glassmeyer
OFFICE: 513-569-7526
FAX: 513-569-7757
glassmeyer.susan@epa.gov

US EPA NERL / MCEARD / CERB
26 W. Martin Luther King Drive
MS 564
Cincinnati, OH 45268 USA

Michael Gonzalez
OFFICE: 513-569-7998
FAX: 513-569-7677
gonzalez.michael@epa.gov

US EPA
26 W. Martin Luther King Drive
MS 443
Cincinnati, OH 45268 USA

Steve Goodbred
OFFICE: 916-278-3097
FAX: 916-278-3071
goodbred@usgs.gov

USGS, Biological Resources Division
California State University
Placer Hall 6000 J. Street
Sacramento, CA 958196129 USA

Jim Goodrich
OFFICE: 513-569-7605
FAX: 513-569-7185
goodrich.james@epa.gov

US EPA WSWRD / NRMRL / ORD
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Denise A. Gordon
OFFICE: 513-569-7594
FAX: 513-569-7609
gordon.denise@epa.gov

US EPA MERB / EERD / NERL
26 W. Martin Luther King Drive
MS 642
Cincinnati, OH 45268 USA

Patricia Grace-Jarrett
OFFICE: 502-540-6145
FAX: 502-540-6365
grace@msdlouky.org

Louisville & Jefferson Co. MSD
700 W. Liberty Street
Louisville, KY 40203 USA

Brian Gullett
OFFICE: 919-541-1534
FAX: 919-541-0290
gullett.brian@epa.gov

US EPA NRMRL
86 Alexander Drive
MD-65
Research Triangle Park, NC 27711 USA

Heldur Hakk
OFFICE: 701-239-1238
FAX: 701-239-1430
hakkh@fargo.ars.usda.gov

USDA-ARS
Biosciences Research Laboratory
P.O. Box 5674 University Station
Fargo, ND 58105-5674 USA

Sharon L. Harper
OFFICE: 919-541-2443
FAX: 919-541-3527
harper.sharon@epa.gov
US EPA
MD-44
Research Triangle Park, NC 27711 USA

Fred Hayden
OFFICE: 510-526-7140
FAX: 510-526-7140
haydens@earthlink.net
Hydrosource
639 Madison Street
Albany, CA 94706 USA

Beverly Head
OFFICE: 513-557-7003
FAX: 513-557-7050
beverly.head@rcc.org
MSD of Greater Cincinnati
1600 Gest Street
Cincinnati, OH 45204 USA

Cynthia Hines
OFFICE: 513-841-4453
FAX: 513-841-4486
chines@cdc.gov
NIOSH
4676 Columbia Parkway
R-14
Cincinnati, OH 45226 USA

Susan Hoertt
OFFICE: 937-384-9940 x3008
FAX: 937-384-9946
sjh@haleyaldrich.com
Haley & Aldrich, Inc.
9039 Springboro Pike
Dayton, OH 45342-4418 USA

Dave Holbrook
OFFICE: 540-953-2870
FAX:
dholbrook@vt.edu
Virginia Tech
418 Durham Hall
Blacksburg, VA 24061 USA

Lindsey Holtgreven
OFFICE: 419-422-3433
FAX:
lholtgr@bsnet.bgsu.edu
Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green , OH 43403 USA

Michael T. Homsher
OFFICE: 419-424-4818
FAX: 419-424-4822
homsher@mail.findlay.edu
University of Findlay
1000 N. Main Street
Findlay, OH 45840 USA

Dan Hopkins
OFFICE: 312-886-5994
FAX: 312-886-2737
hopkins.dan@epa.gov
US EPA
77 W. Jackson Boulevard
Chicago, IL 60604 USA

Steven R. Hutchins
OFFICE: 580-436-8563
FAX: 580-436-8703
hutchins.steve@epa.gov
US EPA NRMRL / SPRD
919 Kerr Research Drive
Ada, OK 74820 USA

Joan M. Jones
OFFICE: 207-287-7879
FAX: 207-287-7826
joan.m.jones@state.me.us
Maine DEP
17 State House Station
Augusta, ME 04333-0017 USA

Jim Kariya
OFFICE: 202-564-8471
FAX: 202-564-8483
kariya.jim@epa.gov
US EPA OPPTS OSCP
1200 Pennsylvania Avenue, NW (MC 7203)
Washington, DC 20460 USA

Hailu Kassa
OFFICE: 419-372-9615
FAX: 419-372-2400
hkassa@bgnet.bgsu.edu
Bowling Green State University
223 Health Center
Ridge Street
Bowling Green, OH 43403 USA

Djanette Khiari
OFFICE: 303-734-3478
FAX: 303-730-0851
dkhiari@awwarf.com
AWWA Research Foundation
6666 W. Quincy Avenue
Denver, CO 80235 USA

Eric J. Kleiner
OFFICE: 513-569-7824
FAX: 513-569-7105
kleiner.eric@epa.gov
US EPA
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Joy R. Klosterman
OFFICE: 937-229-2806
FAX: 937-229-2503
klostejr@udri.udayton.edu
University of Dayton Research Institute
Environmental Sciences & Engineering Group
300 College Park, Kettering Labs, Room 102
Dayton, OH 45469-0132 USA

Vincent J. Kramer
OFFICE: 317-337-3137
FAX: 317-337-4557
vjkramer@dowagro.com
Dow AgroSciences
9330 Zionsville Road
Indianapolis, IN 46268 USA

Fran Kremer
OFFICE: 513-569-7346
FAX: 513-569-7620
kremer.fran@epa.gov
US EPA
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Radha Krishnan
OFFICE: 513-782-4730
FAX: 513-782-4663
rkrishnan@theitgroup.com
IT Corporation
11499 Chester Road
Cincinnati, OH 45246 USA

Jasen M. Kunz
OFFICE:
FAX:
jasenk@bgnet.bgsu.edu
Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Margaret J. Kupferle
OFFICE: 513-569-7548
FAX: 513-569-7108
kupferle.margaret@epa.gov
University of Cincinnati
P.O. Box 210071
Cincinnati, OH 45221-0071 USA

Eugene Langschwager
OFFICE: 513-579-3100
FAX: 513-579-3102
elangsch@gccc.com
Greater Cincinnati Chamber of Commerce
300 Carew Tower
441 Vine Street
Cincinnati, OH 45202-2812 USA

George Lawton OFFICE: 415-467-8779 FAX: eeletter@glawton.com	**Endocrine/Entrogen Letter** P.O. Box 390 Brisbane, CA 94005 USA
Jim Lazorchak OFFICE: 513-569-7076 FAX: 513-569-7609 lazorchak.jim@epamail.epa.gov	**US EPA MERB / EER / NERL** 26 W. Martin Luther King Drive Cincinnati, OH 45268 USA
Linda Lee OFFICE: 765-494-8612 FAX: 765-496-1107 lslee@purdue.edu	**Purdue University** West Lafayette, IN 47907 USA
Peter A. Lewis OFFICE: 513-681-5950 x226 FAX: 513-632-1531 lewispeter@sunchem.com	**Sun Chemical Corporation** 5020 Spring Grove Avenue Cincinnati, OH 45232 USA
Matt Longnecker OFFICE: 919-541-5118 FAX: 919-541-2511	**NIEHS EB** PO Box 12233, MD A3-05 Reserach Triangle Park, NC 27709 USA
Victor Magar OFFICE: 614-424-4604 FAX: 614-424-3667 magarv@battelle.org	**Battelle** 505 King Avenue Columbus, OH 43201 USA
Kathya Mahadevan OFFICE: 513-362-2602 FAX: 513-362-2610 mahadevank@battelle.org	**Battelle Memorial Institute** 655 Eden Park Drive Suite 540 Cincinnati, OH 45202 USA
Todd Martin OFFICE: 513-569-7682 FAX: martin.todd@epa.gov	**ORISE** 26 W. Martin Luther King Drive MS-466 Cincinnati, OH 45242 USA
Robert Masters OFFICE: 614-898-7791 FAX: 614-898-7786 rmaste@ngwa.org	**National Ground Water Association** 601 Demsey Road Westerville, OH 43081 USA
Dr. Eric F. Maurer OFFICE: 513-556-9706 FAX: 513-556-5299 eric.maurer@uc.edu	**University of Cincinnati** 614 Rieveschl Hall Cincinnati, OH 45221 USA
Paul T. McCauley OFFICE: 513-569-7444 FAX: 513-569-7105 mccauley.paul@epa.gov	**US EPA NRMRL / LRPCD / TDB** 26 W. Martin Luther King Drive Cincinnati, OH 45268 USA

Hugh McKinnon
OFFICE: 513-569-7689
FAX: 513-569-7549
mckinnon.hugh@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
MS 225
Cincinnati, OH 45268 USA

Evelyn Meagher-Hartzell
OFFICE: 513-569-5868
FAX: 513-569-4800
evelyn.m.meagher-hartzell@saic.com

SAIC
2260 Park Avenue
Suite 402
Cincinnati, OH 45206 USA

Myriam Medina-Vera
OFFICE: 919-541-5016
FAX: 919-541-3527
Medina-Vera.Myriam@epa.gov

US EPA NERL / HEASD / EMMB
79 T.W. Alexander Drive (MD-44)
Research Triangle Park
Durham, NC 27711 USA

Betty Merriman
OFFICE: 513-569-7454
FAX:
merriman.betty@epa.gov

National Council on Aging
28 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Marc A. Mills
OFFICE: 513-569-7322
FAX: 513-569-7105
mills.marc@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
MS 420
Cincinnati, OH 45268 USA

Dick Miltner
OFFICE: 513-569-7403
FAX: 513-569-7892
miltner.richard@epa.gov

US EPA
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Jami Montgomery
OFFICE: 703-684-2470 x7146
FAX: 703-299-0742
jmontgomery@werf.org

Water Environment Research Foundation
601 Wythe Street
Alexandria, VA 22314 USA

Debdas Mukerjee
OFFICE: 513-569-7572
FAX: 513-569-7475
mukerjee.debdas@epa.gov

US EPA NCEA
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Lee Mulkey
OFFICE:
FAX:
mulkey.lee@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Ron Newhook
OFFICE: 613-957-9576
FAX: 613-954-2486
ron_newhook@hc-sc.gc.ca

Health Canada
EHC, Tunney's Pasture 0802B1
Ottowa, Ontario K1A 0L2 CANADA

Nancy Nilsen
OFFICE: 513-841-4276
FAX: 513-841-4486
nbn9@cdc.gov

NIOSH
5555 Ridge Road
MS R15
Cincinnati, OH 45213 USA

Kazuhiko Nishioka
OFFICE: 212-997-0446
FAX: 212-944-8320
kazuhiko_nishioka@jetro.go.jp
JETRO New York
12221 Avenue of the Americas
New York, NY 10020 USA

Erik Orsak
OFFICE: 702-647-5230
FAX: 702-647-5231
Erik_Orsak@fws.gov
U.S. Fish and Wildlife Service
1510 N. Decatur Boulevard
Las Vegas, NV 89108 USA

Clyde Owens
OFFICE: 919-541-1133
FAX: 919-541-0554
owens.clyde@epa.gov
US EPA APTB
86 Alexander Drive
MD-65
Durham, NC 27709 USA

J. William Owens
OFFICE: 513-627-1385
FAX: 513-627-1208
owens.jw@pg.com
Procter & Gamble
11810 E. Miami River Road
Cincinnati, OH 45252 USA

Neil J. Parke
OFFICE: 317-276-7201
FAX: 317-276-1800
parke_neil_j@lilly.com
Eli Lilly and Company
Lilly Corporate Center
Indianapolis, IN 46285 USA

David Paulsen
OFFICE: 702-450-4440
FAX: 702-454-7966
dpaulsen@co.clark.nv.us
Clark Co. Sanitation District
5857 E. Flamingo Road
Las Vegas, NV 89122 USA

Dan Petersen
OFFICE: 513-569-7831
FAX: 513-569-7585
US EPA
26 W. Martin Luther King Drive
Cincinnati, OH 45208 USA

Frank Pierik
OFFICE: +31 10 408 7448
FAX: +31 10 408 9455
pierik@mgz.egg.eur.nl
Erasmus MC Dept. of Pulic Health
P.O. Box 1738
Rotterdam, 3000 DR THE NETHERLANDS

Fred Pontius
OFFICE: 303-986-9923
FAX: 303-716-7310
fredp@pontiuswater.com
Pontius Water Consultants, Inc.
P.O. Box 150361
Lakewood, CO 80215 USA

Suresh Rao
OFFICE: 765-496-6554
FAX: 765-496-1107
pscr@purdue.edu
Purdue University
School of Civil Engineering
West Lafayette, IN 479071284 USA

Tirumuru V. Reddy
OFFICE: 513-569-7295
FAX: 513-569-7609
reddy.tirumuru@epa.gov
US EPA NERL / EERD / MERB
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Dr. Geeta K. Rijal
OFFICE: 708-588-3767
FAX: 708-588-3807
geeta.rijal@mwrdgc.dst.il.us

Metropolitan Water Reclamation District of Greater Chicago
6001 W. Pershing Road
Cicero, IL 60804 USA

Laura Rosenblum
OFFICE: 513-569-7973
FAX:
rosenblum.laura@epa.gov

IT Corporation
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Ruthann A. Rudel
OFFICE: 617-332-4288
FAX: 617-332-4284
rudel@silentspring.org / elaineruthann@msn.

Silent Spring Institute
29 Crafts Street
Newton, MA 02458 USA

Jeff Ryan
OFFICE: 919-541-1437
FAX: 919-541-0554
ryan.jeff@epa.gov

US EPA APTB
86 Alexander Drive
Durham, NC 27709 USA

Kelly J. Sattler
OFFICE:
FAX:
kellyjs@bgnet.bgsu.edu

Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Gregory Sayles
OFFICE: 513-569-7607
FAX: 513-569-7105
sayles.gregory@epa.gov

US EPA
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Pasquale V. Scarpino
OFFICE: 513-556-3738
FAX: 513-556-2599
pscarpin@uceng.uc.edu

University of Cincinnati
Dept. of Civil & Environmental Engineering
720 Rhodes Hall, M.L. 0071
Cincinnati, OH 45221-0071 USA

Richard A. Scharp
OFFICE: 513-569-7393
FAX: 513-569-7111
scharp.richard@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
Cincinati, OH 45268 USA

Kathleen M. Schenck
OFFICE: 513-569-7947
FAX: 513-569-7185
schenck.kathleen@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Teresa Schnorr
OFFICE: 513-841-4587
FAX: 513-841-4486
tschnorr@cdc.gov

NIOSH
4676 Columbia Parkway
Cincinnati, OH 45226 USA

Dr. Joseph P. Schubauer-Berigan
OFFICE: 513-569-7734
FAX: 513-569-7105
schubauer-berigan.joseph@epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Rachel Sell
OFFICE: 513-362-2605
FAX: 513-362-2610
niemannr@battelle.org

Battelle
Battelle's ETC2
655 Eden Park Drive
Cincinnati, OH 45202 USA

Yonggui Shan
OFFICE: 513-569-7606
FAX: 513-569-7105
shan.yonggui@epa.gov

University of Cincinnati
P.O. Box 210071
Cincinnati, OH 45221 USA

Danielle T. Shirk
OFFICE:
FAX:
shirktk@bgnet.bgsu.edu

Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Kaniz Siddiqui
OFFICE:
FAX:
kaniz.f.siddiqui@rcc.org

MSD City of CIncinnati
1600 Gest Street
Cincinnati, OH 45201 USA

Sukh Sidhu
OFFICE: 937-229-3605
FAX: 937-229-2503
sidhu@udri.udayton.edu

University of Dayton
Environmental Science & English
300 College Park, UDRI, KL102
Dayton, OH 45469-0132 USA

Gary Silverman
OFFICE: 419-372-6062
FAX: 419-372-2400
silverma@bgnet.bgsu.edu

Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Guy Simes
OFFICE: 513-579-7685
FAX: 513-569-7471
simes.guy@epamail.epa.gov

US EPA
26 W. Martin Luther King Drive
Cincinnati, OH 45368 USA

Hugh M. Smith
OFFICE: 513-681-5950 x272
FAX: 513-632-1531
smithh@sunchem.com

Sun Chemical Corporation, Colors Group
5020 Spring Grove Avenue
Cincinnati, OH 45232 USA

Louise T. Snyder
OFFICE: 937-285-6475
FAX:
louise.snyder@epa.state.oh.us

Ohio EPA
401 E. Fifth Street
Dayton, OH 45402 USA

Adam Socha
OFFICE: 416-327-3912
FAX: 416-327-9091
adam.socha@ene.gov.on.ca

Ontario Ministry of the Environment
Standards Development Branch
125 Resources Road, North Wing
Etobicoke, Ontario M9P 3V6 CANADA

Thomas Speth
OFFICE: 513-569-7208
FAX: 513-569-7892
speth.thomas@epa.gov

US EPA
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Sara Spino
OFFICE:
FAX:
sspino@bgnet.bgsu.edu

Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Laurel Staley
OFFICE: 513-569-7863
FAX: 513-569-7105
staley.laurel@epamail.epa.gov

US EPA NRMRL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

T. R. Steinheimer
OFFICE: 515-294-2952
FAX: 515-294-8125
steinheimer@nstl.gov

USDA ARS
National Soil Tilth Laboratory
2150 Pammel Drive
Ames, IA 50011-4047 USA

Tammy Stoker
OFFICE: 919-541-2783
FAX: 919-541-5138
stoker.tammy@epa.gov

US EPA NHEERL / GEEB
2525 Alexander Drive
MD-72
Research Triangle Park, NC 27713 USA

Gary Strassell
OFFICE: 513-874-0714
FAX: 513-874-5061
gstrassell@shepherdcolor.com

The Shepherd Color Company
PO Box 465627
Cincinnati, OH 45246 USA

Richard C. Striebich
OFFICE: 937-229-2847
FAX: 937-229-2503
striebich@udri.udayton.edu

University of Dayton Research Institute
300 College Park
KL 102
Dayton, OH 45469-0132 USA

Daniel Sullivan
OFFICE: 616-833-0394
FAX:
daniel.e.sullivan@pharmacia.com

Pharmacia Corporation
7000 Portage Road
MS 6605-88-009
Kalamazoo, MI 49001 USA

Kathryn Tierney
OFFICE: 003-2229-68118
FAX: 003-2229-91067
kathryn.tierney@cec.eu.int

Europeon Commission
200 Rue De La Loi (B45 2/157)
Brussels, Belguium 1049 Europe

Greg Toth
OFFICE: 513-569-7242
FAX: 513-569-7609
toth.greg@epa.gov

US EPA NERL
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Marcus Tsilimos
OFFICE:
FAX:
marcust@bgnet.bgsu.edu

Bowling Green State University
Environmental Health Program
223 Health Center
Bowling Green, OH 43403 USA

Raghuraman Venkatapathy
OFFICE: 513-569-7077
FAX: 513-569-7475
venkatapathy.raghuraman@epa.gov

US EPA NCEA / ORISE
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Karl G. Voelkel
OFFICE: 937-259-5041
FAX: 937-259-5100
kvoelkel@ljbinc.com

LJB, Inc.
3100 Research Boulevard
Dayton, OH 45420-0246 USA

Mark A. Walton
OFFICE: 208-938-5130
FAX: 208-938-5662
mawalton@dow.com

The Dow Chemical Company
1924 S. Riverford Place
Eagle, ID 83616USA

David Warshawsky
OFFICE: 513-558-0152
FAX:

University of Cincinnati
P.O. Box 210071
Cincinnati, OH 45221-0071 USA

Martha J. M. Wells
OFFICE: 931-372-3507
FAX: 931-372-6346
mjmwells@tntech.edu

Tennessee Technological University
P.O. Box 5033
1020 Stadium Drive
Cookeville, TN 38505 USA

Dennis J. Wesolowski
OFFICE: 312-353-9084
FAX: 312-886-2591
wesolowski.dennis@epa.gov

US EPA Region 5
536 S. Clark Street
Chicago, IL 60605 USA

Elizabeth Whelan
OFFICE: 513-841-4437
FAX: 513-841-4486
EWhelan@cdc.gov

National Institute for Occupational Safety and Health
4676 Columbia Parkway
R-15
Cincinnati, OH 45226 USA

Greg Woodside
OFFICE: 714-378-3275
FAX: 714-378-3369
gwoodside@ocwd.com

Orange County Water District
10500 Ellis Avenue
Fountain Valley, CA 92626 USA

Michael Wright
OFFICE: 513-569-7922
FAX: 513-569-7475
wright.michael@epa.gov

ORISE-NCEA
140 Warner Street
#1
Cincinnati, OH 45219 USA

Douglas Young
OFFICE: 513-569-7624
FAX: 513-569-7111
young.douglas@epa.gov

US EPA ORD / NRMRL / STD / SAB
26 W. Martin Luther King Drive
Cincinnati, OH 45268 USA

Diana Zimmerman
OFFICE: 937-285-6440
FAX: 937-285-6249
diana.zimmerman@epa.state.oh.us

Ohio EPA
401 E. Fifth Street
Dayton, OH 45402 USA

Lawrence Zintek
OFFICE: 312-886-2925
FAX: 312-886-2591
zintek.lawrence@epa.gov

US EPA Region 5
77 W. Jackson Boulevard
MC ML-10C
Chicago, IL 60605 USA

Effective Risk Management of Endocrine Disrupting Chemicals

January 29-30, 2002

Cincinnati, Ohio

Sponsored by

U.S. Environmental Protection Agency
Office of Research and Development
National Risk Management Research Laboratory

Effective Risk Management of Endocrine Disrupting Chemicals

Vernon Manor Hotel
Cincinnati, OH
January 29-30, 2002

Final Agenda

☎ Tuesday, January 29, 2002☎

7:00-8:20 AM Registration

8:30 AM *Welcome and Logistics*
John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

Risk Management Context
Moderator: Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

8:35 AM *Purpose and Goals of the Workshop*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

8:40 AM *Welcome from the National Risk Management Research Labora*tory
Lee Mulkey, U.S. EPA, National Risk Management Research Laboratory

8:50 AM *Risk Management Research: Improving Environmental Decisions*
Hugh McKinnon, U.S. EPA, National Risk Management Research Laboratory

9:20 AM *European Community Strategy for Endocrine Disruptors: Implementation to Date*
Kathryn Tierney, European Commission

9:50 AM Break

Effects of EDCs on Humans and Wildlife
Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

10:10 AM *Introduction to EDCs and Their Potential Effects on Humans*
Ralph Cooper, U.S. EPA, National Health and Environmental Effects Laboratory

10:50 AM *Overview of Effects and Assessment of Endocrine-Disrupting Chemicals in Wildlife*
Gary Ankley, U.S. EPA, National Health and Environmental Effects Laboratory

11:30 AM *EPA's Endocrine Disruptors Screening Program: Legislation, Implementation, and Research*
Elaine Francis, U.S. EPA, National Center for Environmental Research

12:00 PM Lunch

Exposure Assessment for EDCs
Moderator: John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

1:10 PM *Development of Biological Methods to Characterize Exposure of Wildlife to EDCs*
Greg Toth, U.S. EPA, National Exposure Research Laboratory

1:40 PM *Development of Chemical Methods to Characterize Exposure to EDCs in the Neuse River Basin*
Myriam Medina-Vera, U.S. EPA, National Exposure Research Laboratory

2:10 PM *Monitoring Endocrine Disrupting Compounds in Aquatic Ecosystems in the United States*
Steve Goodbred, U.S. Geological Survey

2:40 PM *Region 5: Ongoing Endocrine Disruptor Efforts*
Lawrence Zintek, U.S. EPA, Region 5

3:10 PM Break

3:30 PM *Residential Indoor Air and Dust Measurements of Phthalates and Other Endocrine Disrupting Compounds*
Ruthann Rudel, Silent Spring Institute

Risk Management Approaches
Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

4:00 PM *EPA's Risk Management Evaluation of EDCs*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

4:40 PM *Using Bioassays to Evaluate the Performance of Risk Management Techniques*
Carolyn Acheson, U.S. EPA, National Risk Management Research Laboratory

5:10 PM Adjourn for the day

☎ Wednesday, January 30, 2002 ☎

8:30 AM *Welcome and Logistics for Day 2*
John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

Drinking Water Treatment
Moderator: James Goodrich, U.S. EPA, National Risk Management Research Laboratory

8:35 AM *Use of Granular Activated Carbon and Powdered Activated Carbon for the Removal of EDCs from Drinking Water: A User's Guide*
John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

9:05 AM *Evaluation of Drinking Water Treatment Technologies for Removal of Endocrine Distrupting Compounds*
Kathleen Schenck, U.S. EPA, National Risk Management Research Laboratory

9:20 AM *Risk Management of Endocrine Disrupting Chemicals in Drinking Water*
Frederick Pontius, Pontius Water Consultants, Inc.

9:50 AM Break

Concentrated Animal Feed Operations
Moderator: Laurel Staley, U.S. EPA, National Risk Management Research Laboratory

10:10 AM *Potential of Confined Animal Feed Operations (CAFOs) to Contribute Estrogens to the Environment*
Steven Hutchins, U.S. EPA, National Risk Management Research Laboratory

10:40 AM *Investigations of Sorption and Transport of Hormones and Animal Pharmaceuticals: Initial Laboratory Results*
Suresh Rao, Purdue University
Linda Lee, Purdue University

11:00 AM *Fate of the Endogenous Hormones 17ß-Estradiol and Testosterone in Composted Poultry Manure and their Sorption and Mobility in Loam Soil and Sand*
Heldur Hakk, USDA, Agricultural Research Service

11:30 AM Lunch

Wastewater Treatment

Moderator: Marc Mills, U.S. EPA, National Risk Management Research Laboratory

12:45 PM *Biological Fate of Estrogenic Compounds Associated with Sewage Treatment*: *A Review*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

1:15 PM *An Engineering Approach to Evaluate Estrogenic EDC's Fate During Wastewater Treatment*
Paul McCauley, U.S. EPA, National Risk Management Research Laboratory

1:30 PM Break

Other EDC Risk Management Challenges
Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

1:45 PM *Endocrine Disruptors from Combustion and Vehicular Emissions: Identification and Source Nomination*
Brian Gullett, U.S. EPA, National Risk Management Research Laboratory

2:00 PM *Natural Recovery of PCB-Contaminated Sediments at the Sangamo-Weston / Twelve Mile Creek / Lake Hartwell Superfund Site*
Richard Brenner, U.S. EPA, National Risk Management Research Laboratory
James Lazorchak, U.S. EPA, National Exposure Research Laboratory

2:45 PM *Program for the Identification and Replacement of Endocrine Disrupting Chemicals*
Douglas Young, U.S. EPA, National Risk Management Research Laboratory

3:00 PM Adjourn Workshop

Updated January 18, 2002

TABLE OF CONTENTS

☎ Tuesday, January 29, 2002 ☎

Risk Management Context

Moderator: Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

8:35 AM *Purpose and Goals of the Workshop*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

8:40 AM *Welcome from the National Risk Management Research Labora*tory
Lee Mulkey, U.S. EPA, National Risk Management Research Laboratory

8:50 AM *Risk Management Research: Improving Environmental Decisions*
Hugh McKinnon, U.S. EPA, National Risk Management Research Laboratory

9:20 AM *European Community Strategy for Endocrine Disruptors: Implementation to Date*
Kathryn Tierney, European Commission

9:50 AM Break

WELCOME FROM THE NATIONAL RISK MANAGEMENT RESEARCH LABORATORY

Lee Mulkey
U.S. Environmental Protection Agency
National Risk Management Research Laboratory
26 West Martin Luther King Drive
Cincinnati, OH 45268
513-569-7689
mulkey.lee@epa.gov

THE SPEAKER'S ABSTRACT IS UNAVAILABLE, PLEASE SEE THE SPEAKER WITH COMMENTS AND/OR QUESTIONS.

Lee A. Mulkey

Lee A. Mulkey is the Associate Director of Ecology at the U.S. EPA's National Risk Management Research Laboratory in Cincinnati, OH. He is responsible for developing and providing science policy guidance for EPA's research on protecting ecosystems. His professional experience has focused on watershed management research, including: modeling, best management practice development and testing, and risk assessment.

RISK MANAGEMENT RESEARCH: IMPROVING ENVIRONMENTAL DECISIONS

Hugh W. McKinnon, M.D., M.P.H.
U.S. Environmental Protection Agency
National Risk Management Research Laboratory
26 West Martin Luther King, Jr. Drive
Cincinnati, OH 45268
(513) 569-7689
(513) 569-7549 fax
mckinnon.hugh@epa.gov

Over the past two decades much progress has been made in defining and implementing the disciplines and processes of risk assessment, including research to reduce uncertainties in risk assessment. Similar definition is needed for the risk management process, and risk management research is needed to provide this definition and reduce uncertainties in risk management. This presentation will describe some of the needs and issues associated with risk management research, including creation of risk management evaluations (RME) and a protocol for developing or conducting risk management evaluations.

Hugh W. McKinnon, M.D., M.P.H.

Dr. Hugh McKinnon is a Senior Executive Medical Officer in the U.S. Environmental Protection Agency (EPA) and is the Associate Director for Health in EPA's National Risk Management Research Laboratory (NRMRL) in Cincinnati, Ohio, a position he has held since May 1995. From 1989 to 1995 Dr. McKinnon was Director of EPA's Human Health Assessment Group in Washington, DC, which produced EPA's January 1993 report on respiratory effects, including lung cancer, associated with exposure to environmental tobacco smoke (ETS). Earlier Dr. McKinnon served for ten years as a Medical Officer in EPA's Office of Health Research, also in Washington, DC, and was the Acting Director of that office from 1985 to 1987. He received a Doctor of Medicine (M.D.) Degree from the University of Virginia in 1977 and a Master of Public Health (M.P.H.) Degree from The John Hopkins University, where he completed the residency in General Preventive Medicine in 1989. He has also had clinical training in general surgery and in family practice. Dr. McKinnon is a member of and serves on committees and boards of the American College of Occupational and Environmental Medicine (ACOEM), the American Public Health Association (APHA), and the Federal Physicians Association. He also serves as the EPA representative to the National Cancer Advisory Board of the National Cancer Institute.

EUROPEAN COMMUNITY STRATEGY FOR ENDOCRINE DISRUPTERS: IMPLEMENTATION TO DATE

Kathryn Tierney
European Commission, Environment Directorate-General
200 Rue de la Loi (BU5 2/157), B-1049 Brussels, Belgium
Tel +32-2-296 8118; Fax +32-2-299 1067; kathryn.tierney@cec.eu.int

In December 1999, the European Commission adopted a Communication to the Council and European Parliament on a Community Strategy for Endocrine Disrupters (COM(1999)706). The strategy addresses the key requirements of further research, international co-operation, communication to the public, and appropriate policy action. Recommendations are made for short-, medium- and long-term actions.

In March 2000, the Environment Council of Ministers adopted Conclusions on the Commission Communication in which it stressed the precautionary principle, the need to develop quick and effective risk management strategies and the need for consistency with the overall EU chemical policy.
In October 2000, the European Parliament adopted a Resolution on endocrine disrupters, emphasising the application of the precautionary principle and calling on the European Commission to identify substances for immediate action.

A key short-term action of the Community Strategy is the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. During 2000, a candidate list of 553 man-made substances and 9 synthetic/natural hormones was identified and a priority list of actions has been developed in order to further evaluate the role of these substances in endocrine disruption.
In June 2000, the European Commission organised a European Workshop on Endocrine Disrupters in Sweden, which focused on information exchange and international co-operation, research and development, test methods/testing strategy and establishment of monitoring programmes.

The Commission and Member States continue to participate in the OECD Endocrine Disrupter Testing and Assessment Task Force, which was set up in 1998 with the goal of developing agreed test methods for endocrine disrupters.

Under the 5th Community Framework Programme for R&D (1999-2002), research into endocrine disruption has been prioritised. In May 2001, a dedicated call for research proposals on the health and environmental implications of endocrine disrupters was published with a budgetary envelope of 20 MEURO.
Finally, regarding legislative action, the revision of the General Product Safety Directive will lead, inter-alia, to a simplification of conditions and procedures for urgent measures at Community level. In addition, the issue of endocrine disrupters is addressed specifically in the context of new and existing legislation in the field of water policy and in the White Paper on a strategy for a future chemicals policy.

REFERENCE

Communication from the Commission to the Council and European Parliament on the implementation of the Community Strategy for Endocrine Disrupters (COM(2001)262).

Kathryn Tierney

Kathryn Tierney is a Principal Administrator with the European Commission. She has a Bachelor's Degree in Industrial Engineering and a Master's Degree in Engineering Science from the National University of Ireland. She joined the European Commission in 1991 and first worked on the establishment of an international research programme involving Australia, Canada, Japan, the USA and the EU/EFTA countries. In 1997 she joined the Environment Directorate-General and has been working on the topic of endocrine disruption from that time. Before joining the Commission, Ms. Tierney worked for Digital Equipment Corporation in Ireland and the US and for the Irish Science and Technology Agency.

☎ Tuesday, January 29, 2002 ☎

Effects of EDCs on Humans and Wildlife

Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

10:10 AM *Introduction to EDCs and Their Potential Effects on Humans*
Ralph Cooper, U.S. EPA, National Health and Environmental Effects Laboratory

10:50 AM *Overview of Effects and Assessment of Endocrine-Disrupting Chemicals in Wildlife*
Gary Ankley, U.S. EPA, National Health and Environmental Effects Laboratory

11:30 AM *EPA's Endocrine Disruptors Screening Program: Legislation, Implementation, and Research*
Elaine Francis, U.S. EPA, National Center for Environmental Research

12:00 PM Lunch

INTRODUCTION TO EDCs AND THEIR POTENTIAL EFFECTS ON HUMANS

Ralph L. Cooper, Chief
Endocrinology Branch, Reproductive Toxicology Division, National Health and Environmental Effects Research Laboratory, U.S. EPA
Research Triangle Park, NC 27711
(919) 541-4084, (919)541-5138 fax
cooper.ralph@epa.gov

The endocrine system provides important regulatory support to the homeostatic mechanisms involved in a variety of physiological functions. These precisely timed events require appropriate signaling between the various organs which includes the synthesis, release, transport identification and clearance of individual hormones associated with such complex processes as puberty, reproduction, adaptation to stress, normal metabolic function, and behavior. As such, the endocrine system provides a number of target sites that may be susceptible to disruption by environmental agents. The purpose of this discussion will be to provide a brief overview of the hormonal control of reproductive and thyroid function including, a description of the organs and hormones involved. This will be followed by a summary of the cellular mechanisms of primary concern to the issue of endocrine disruption such as the enzymes involved in the synthesis of hormones, nuclear and membrane receptors, and the processes involved in metabolism (clearance). The issue of endocrine disruptors will then be discussed focusing on those reports that have raised concern that environmental endocrine disruptors may be responsible for impaired human reproductive health. This discussion will be followed by a review of the ongoing efforts of EPAs Endocrine Disruptor Screening Program as it relates to evaluating chemicals for their potential endocrine disrupting effects on mammalian reproduction, reproductive development and thyroid function.

Ralph Cooper

Dr. Ralph Cooper is the Chief of the Endocrinology Branch at the U.S. EPA National Health and Environmental Effects Laboratory (NHEERL) Reproductive Toxicology Division (RTD) (1995-present). Dr. Cooper was the chief of Endocrinology/Gerontology Section at NHEERL RTD from 1984-1995. He has a Ph.D. in Psychobiology from Rutgers University and a NIH Post Doctorate from Duke University's Neuroscience Program. Dr. Cooper is currently the Chair of the NHEERL Endocrine Disruptor Research Implementation Committee (2000-present). Dr. Cooper co-chaired the RTD/Program Office and Region Workshop on Emerging Issues in Reproductive Toxicology in June 2001. Dr. Cooper is also a member of Sigma Xi, the North Carolina Chapter of the Society for Neuroscience, and the Endocrine Society. He is the recipient of numerous awards including: 1) Best Paper Published in Reproductive & Developmental Toxicology Specialty Section, Toxicological Sciences (2001); 2) the EDSP team award for Exceptional/Outstanding ORD Technical Assistance to the Regions or Program Offices (2001); 3) the Office of Pesticides Programs Health Effects Division Team Award for work related to the chlorotriazines (2000); 4) Best Paper Published in Reproductive & Developmental Toxicology Specialty Section, Toxicological Sciences (1999); and 5) a Bronze Medal ORD/RAF Workgroup on Environmental Endocrine Disruption: An Effects Assessment and Analysis Document (1998).

OVERVIEW OF EFFECTS AND ASSESSMENT OF ENDOCRINE-DISRUPTING CHEMICALS IN WILDLIFE

Gary Ankley
U.S. Environmental Protection Agency
Environmental Effects Research Laboratory
6201Congdon Blvd.
Duluth, MN 55804
(218) 529-5147
ankley.gerald@epa.gov

THE SPEAKER'S ABSTRACT IS UNAVAILABLE, PLEASE SEE THE SPEAKER WITH COMMENTS AND/OR QUESTIONS.

Gerald T. (Gary) Ankley

Dr. Ankley is the Chief of the U.S. EPA Mid-Continent Ecology Division, Toxic Effects Characterization Branch. He is also an adjunct professor in the Department of Biology at Michigan Technological University and the Department of Fisheries and Wildlife at the University of Minnesota. Dr. Ankley is the Aquatic Toxicology Editor for Environmental Toxicology and Chemistry and is a member of the Editorial Board for Chemosphere. He has a Ph.D. and MS in Forest Resources (Aquatic Toxicology) from the University of Georgia. Dr. Ankley has received numerous awards, including: fourteen EPA awards in recognition of different aspects of leadership, scientific planning, and contributions to specific research projects; his manuscript was nominated for best paper in the 2000 volume of North America Journal of Aquaculture; and the Scientific and Technology Achievement Award (STAA) for journal articles on development and application of chronic sediment test with *Chironomus tentans*.

EPA's ENDOCRINE DISRUPTORS SCREENING PROGRAM: LEGISLATION, IMPLEMENTATION, AND RESEARCH

Elaine Z. Francis
U.S. Environmental Protection Agency
Office of Research and Development/National Center for Environmental Research
1200 Pennsylvania Avenue, NW (8701R)
Washington, DC 20460
(202) 564-6789
(202) 565-2444 fax
francis.elaine@epa.gov

In 1996, the Food Quality Protection Act (FQPA) and the Safe Drinking Water Act Amendments (SDWAA) were enacted and specifically require the testing of pesticides and other chemicals found in or on food or in drinking water sources to determine their "estrogenic or other endocrine effects in humans." These laws required EPA to develop a screening program by August 1998, to implement the program by August 1999, and to report to Congress on the program's progress by August 2000. The Agency sought advice on how to design such a program by convening the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), a 39-member panel representing broad constituencies. EDSTAC issued a final report in August 1998 that included 71 specific recommendations (*www.epa.gov/scipoly/oscpendohistory/)*. Among the key recommendations were that EPA's endocrine disruptors screening program (EDSP) should be expanded beyond the provisions of FQPA, taking into consideration other EPA mandates and, thus, should: 1) address both human and ecological (wildlife) effects, 2) examine effects to not only estrogen but also androgen and thyroid processes, and 3) evaluate endocrine disrupting properties of chemicals and common mixtures. EDSTAC recommended a tiered approach that included: initial sorting, priority setting, Tier 1 screening and Tier 2 testing. They recommended a battery of eight *in vitro* and *in vivo* assays for Tier 1 screening with four additional assays as alternatives. Tier 2 testing recommendations included multi-generation studies conducted in mammalian, avian, fish, invertebrate, and amphibian species. EPA's Office of Prevention, Pesticides, and Toxic Substances has developed a program to implement the legislative mandates taking into consideration the EDSTAC recommendations. They have held a series of public meetings and workshops on various aspects of the program. The report to Congress issued August 2000 describes EPA's implementation of the science-driven EDSP. Implementation activities include completing the Endocrine Disruptor Priority Setting Database and the compartment-based approach, that the Agency will use to establish priorities for screening chemicals at a later stage of implementation, and ensuring that the Tier 1 screens and Tier 2 tests are validated as required by statute. Research conducted by EPA's Office of Research and Development (ORD) is addressing the scientific questions that have arisen as a result of the FQPA and SDWAA and is leading to the development of protocols that will undergo validation for the EDSP. The screens will be completed by 2003 and the tests by 2005. In addition, ORD's long-term research program on ED focuses on the most critical uncertainties in determining whether humans and wildlife are being impacted by levels of ED in the environment, in identifying the sources of those exposures, developing approaches to integrate information into risk assessments, and developing risk management approaches to reduce/prevent exposures.

Elaine Z. Francis

Dr. Elaine Francis has been with EPA for 21 years – first as a developmental/ reproductive toxicologist with the Office of Toxic Substances and, since 1988, with the Office of Research and Development. She spent 1991 as a legislative fellow to Senator Joseph Lieberman working on pesticides, lead, and children's issues. From 1995 to 2000 she was the Pesticides, Toxics, and Multimedia Staff Director and coordinated the planning for many of ORD's research programs including those for toxics and pesticides, human health, ecological risk assessment, pollution prevention, and endocrine disruptors. In January 2000, Elaine became the National Program Director for EPA's endocrine disruptors research program. She received her doctorate in Anatomy from Thomas Jefferson University, where the focus of her training and research was on normal and abnormal human development and reproduction.

☎ Tuesday, January 29, 2002☎

Exposure Assessment for EDCs

Moderator: John Cicmanec, U.S. EPA, National Risk Management Research Laboratory

1:10 PM *Development of Biological Methods to Characterize Exposure of Wildlife to EDCs*
Greg Toth, U.S. EPA, National Exposure Research Laboratory

1:40 PM *Development of Chemical Methods to Characterize Exposure to EDCs in the Neuse River Basin*
Myriam Medina-Vera, U.S. EPA, National Exposure Research Laboratory

2:10 PM *Monitoring Endocrine Disrupting Compounds in Aquatic Ecosystems in the United States*
Steve Goodbred, U.S. Geological Survey

2:40 PM *Region 5: Ongoing Endocrine Disruptor Efforts*
Lawrence Zintek, U.S. EPA, Region 5

3:10 PM Break

3:30 PM *Residential Indoor Air and Dust Measurements of Phthalates and Other Endocrine Disrupting Compounds*
Ruthann Rudel, Silent Spring Institute

DEVELOPMENT OF BIOLOGICAL METHODS TO CHARACTERIZE EXPOSURE OF WILDLIFE TO EDCs

Greg Toth
U.S. Environmental Protection Agency
NERL EERD MER
26 W. Martin Luther King, Jr. Drive
Cincinnati, OH 45268
(513) 569-7242
(513) 569-7609 fax
toth.greg@epa.gov

Biological indicators of exposure are being developed to monitor surface and ground water samples for the presence of extrogenic activity. Changes in vitellogenin gene expression in the livers of adult male fathead minnows (Pimephales promelas) have been studied in controlled laboratory, mesocosm and field studies. In the laboratory, following static exposure of fatheads to 17”-ethinyl estradiol for 24 hours, vitellogenin gene expression was detected at 2 ng/l. Expression has also been detected in the gills of adult fatheads as well as in 24 hour whole larva. This indicator method has been used in surrogate ecosystems (mesocosms) to study the attenuation of synthetic estrogens across differing nutrient status. Field study includes the monitoring of risk management practices (e.g., sediment capping) to evaluate their effectiveness by using similar molecular assays – changes in fathead minnow liver cytochrome P450IA1 levels. Current biological indicator research includes the development of methods to diagnose the presence of multiple chemical stressors in mixtures using advanced molecular methods – DNA microarrays.

Gregory P. Toth

Dr. Toth is the Branch Chief of the Molecular Ecology Research Branch at the U.S. EPA National Exposure Research Laboratory (NERL) Ecological Exposure Research Division (EERD) in Cincinnati, OH. He has a Ph.D. in Biological Chemistry from the University of Cincinnati College of Medicine, Department of Biological Chemistry (1982) and a BS in Chemistry from John Carroll University (1975). Dr. Toth was an Albert J. Ryan Fellow (1976-1981) and received a National Research Council Research Associateship (1985-1986). Dr. Toth has been published in numerous journals and publications including: Molecular Ecology, Electrophoresis, the American Journal of Botany, and Environmental Toxicology and Chemistry.

DEVELOPMENT OF CHEMICAL METHODS TO CHARACTERIZE EXPOSURE TO EDCS IN THE NEUSE RIVER BASIN

Myriam Medina-Vera
U.S. Environmental Protection Agency
NERL HEASD EMMB
79 T.W. Alexander Drive (MD-44)
Research Triangle Park
Durham, NC 27711
(919) 541-5016
(919) 541-3527 fax
Medina-Vera.Myriam@epa.gov

To develop a quantitative health and environmental risk assessment of endocrine disrupting compounds (EDCs), information on exposures is essential. A full exposure assessment has complex requirements that require preliminary information to direct further research in this area. Such research begins with refining the biological and chemical methods for selected endocrine disrupting compounds in fresh and brackish, polluted and unpolluted waters, sediment, soil and selected aquatic organisms. Accurate characterization of the exposures can be done by using valid methods that are sensitive and reliable. Adequate methods provide tools that will help with the understanding of pathways of exposure, fate and transport of selected endocrine disruptors. Identification of data gaps stressed the need for better methods in the identification and quantitation of EDCs such as alkylphenols and selected toxic metals.

Myriam Medina-Vera

Dr. Medina-Vera has a BS in Chemistry and Mathematics (double major) and a Ph.D. in Analytical Chemistry with minors in Physical Chemistry and Inorganic Chemistry from the University of Puerto Rico. She has been working for EPA since 1991. Her research areas include Photochemistry of PAHs on particulate matter, Pyrolysis of PAHs, and analysis alkylphenols. She is currently the chief of NERL's Exposure Methods and Monitoring Branch in RTP.

Monitoring Endocrine Disrupting Compounds in Aquatic Ecosystems in the United States

Steven L. Goodbred,
U.S. Geological Survey, Placer Hall, 6000 J Street
California State University
Sacramento, CA 95821
(916) 278-3097
goodbred@usgs.gov

Several Federal Agencies have monitoring programs that include suspected endocrine disrupting compounds (EDCs); however no systematic monitoring of only EDCs is routinely done in the United States. The U.S. Geological Survey's National Water Quality Assessment Program (NAWQA) was established to assess status and trends of surface-water and ground-water throughout streams, rivers, and aquifers of the US (Gilliom et al. 1995). Fourty-four EDCs have been monitored in water, bed sediment, and fish since 1991 at 1363 sites. Results have shown several suspected EDCs like atrazine are widespread in US streams and their occurrence appears related to land and chemical use. Some EDCs like PCBs are more frequently detected and at higher concentrations in urban streams while others like *p,p'* DDE in fish are found in much higher concentration in agricultural land use. At many sites the detection of breakdown products and metabolites was 10 to 25 times the concentration of the parent compound.

Although pesticides are at low levels (<ug/L) they are almost always present as mixtures making risk assessments more difficult. Another difficulty with conducting a national scale risk assessment is exposure. Results from temporal sampling during pesticide application shows pesticide occurrence in streams in strong seasonal pulses and is related to climate and runoff. This kind of exposure to EDCs is very difficult to mimic in the laboratory and just as difficult to model. Field studies of EDC effects can help address these issues. Results of a USGS National Reconnaissance of potential endocrine disruption in fish showed a strong correlation of dissolved pesticides and hormone ratios in both male and female fish (Goodbred et al. 1997). A reduced ratio of estrogens to androgens in female fish from sites like the Platte River at Louisville NE, could affect reproduction.

Another USGS water quality study that will have information on occurrence of EDCs is the Toxicis Program's National Reconnaissance of Pharmaceuticals and other Emerging Contaminants in US Streams. The goal is to determine if pharmaceuticals, antibiotics, hormones, and other wastewater compounds are entering the environment. They have developed new analytical methods to detect these compounds in water and bed sediment that include quite a few EDCs, like bisphenol A, 17*a* ethynyl estradiol, and testosterone. Other compounds like galaxolide a musk derivative used in fragrances, and triclosan a common antimicrobial have the potential to be either an EDC or have some other effects on orgainisms but need to be studied further (Daughton and Ternes, 1999). In 1999/2000 143 streams in 32 states were sampled for 98 compounds. Results are being published later this year and will be available at this web site: toxics@usgs.gov.

References:

Daughton, C., T. Ternes, 1999, Pharmaceuticals and personal care products in the environment: agents of subtle change? Environmental Health Perspectives, vol. 107, Supplement 6, p.907-938

Gilliom, R., W.Alley, and M.Gurtz, 1995, Design of the National Water Quality Assessment Program- Occurrence and distribution of water-quality conditions: U.S. Geological Survey Circular 1112, 33p.

Goodbred, S., R.Gilliom, T.Gross, N. Denslow, W.Bryant, and T.Schoeb, 1997, Reconnaissance of 17*B*-estradiol, 11-ketotestosterone, vitellogenin, and gonad histopathology in common carp of United States streams-Potential for contaminant-induced endocrine disruption: U.S. Geological Survey Open File Report 96-627, 47p.

THE SPEAKER'S BIO IS UNAVAILABLE. PLEASE SEE THE SPEAKER WITH COMMENTS AND/OR QUESTIONS

REGION 5: ONGOING ENDOCRINE DISRUPTOR EFFORTS

Peter Howe, Al Alwan, John Dorkin, George Azevedo, Mari Nord, Marc Tuchman, Dennis Wesolowski, Babu Paruchuri, Lawrence Zintek*

U.S. EPA Region 5
77 West Jackson Blvd.
Chicago, IL 60604
(312) 353-9067
(312) 886-2591 Fax
zintek.lawrence@epa.gov

Region 5 efforts are focused on the alkylphenols such as; nonylphenol, nonylphenol ethoxylates, nonylphenol carboxylates and octylphenol. Our interest is to discover if these chemicals are present in our region and if so are they present at a concentration harmful to the environment. The region was determined to have the need for standards of known purity fulfilled by getting them synthesized and made commercially available. The material presented includes several studies demonstrating that effluent concentrations of chemicals of interest are above the effect level in water bodies receiving effluent from Publicly Owned Treatment Works (POTW=s). Water, sediment and fish samples were taken and analyzed from different river systems in Region 5. Some data were collected through funding of studies by USGS and USDA.

Lawrence Zintek

Dr. Lawrence Zintek is with the U.S. EPA Region 5, Central Regional Laboratory. Prior to working at the U.S. EPA, he was an instructor at Benedictine University from 1995-2000. Dr. Zintek has a Ph.D. in Chemistry from the University of Iowa and a BS in Biochemistry from Illinois Benedictine College.

RESIDENTIAL INDOOR AIR AND DUST MEASUREMENTS FOR PHTHALATES AND OTHER ENDOCRINE DISRUPTING COMPOUNDS

Ruthann Rudel[1*], Julia G. Brody[1], David Camann[2], Alice Yau[2], John D. Spengler[3]

[1]Silent Spring Institute, 29 Crafts Street, Newton, MA 02458
(617) 332-4288 x14, (617) 332-4284 fax
Rudel@SilentSpring.org
[2]Southwest Research Institute, 6220 Culebra Road,PO Drawer 28510
San Antonio, TX 78228
(210) 522-5947, (210) 522-5938 fax
[3]Harvard School of Public Health, 665 Huntington Ave,Boston, MA 02115
(617) 432-1255, (617) 432-4122 fax
jspengle@hsph.harvard.edu

In order to characterize exposures to chemicals of interest for research on breast cancer and other hormonally mediated health outcomes, residential air and dust samples were analyzed for up to 93 target compounds that 1) have been identified as animal mammary carcinogens or hormonally active chemicals and 2) are used in commercial or consumer products or building materials. Selected phthalates, pesticides, parabens, PAHs and PCBs were extracted and analyzed by GC/MS-SIM. Phenolic compounds including nonylphenol, octylphenol, bisphenol A, and the methoxychlor metabolite HPTE were extracted, derivatized, and analyzed by GC/MS-SIM (Rudel et al., 2001). In data from the first 30 of 120 homes sampled on Cape Cod, MA, 44 of 68 target compounds were detected in at least one air sample and 68 of 93 were detected in at least one dust sample.

In these 30 air samples, diethyl phthalate was detected at the highest concentrations (range 128-2,113 ng/m^3), followed by dibutyl phthalate (56-851 ng/m^3), di (2-ethylhexyl) phthalate (DEHP) (57-562 ng/m^3) and diisobutyl phthalate (18-986 ng/m^3). Six other phthalates were also detected. It is of interest that the most abundant phthalates in indoor air are also most abundant in human urine samples collected by CDC for a reference population of US adults (CDC 2001). Other EDCs present in air at highest concentrations included nonylphenol (48-416 ng/m^3) and the disinfectant o-phenyl phenol (43-958 ng/m^3). Pesticides detected in over half the air samples tested included propoxur, chlordane, pentachlorophenol, heptachlor, diazinon, and chlorpyrifos.

In 30 dust samples, the most widely used phthalates were most abundant (NTP-CEHR 2000). Thus DEHP was present at highest concentrations (range 125-1082 ?g/g), followed by BBP (9-749 ?g/g) and diisononyl phthalate (<DL-785 ?g/g). Nonylphenol was detected in all dust samples (range 1.6-7 ?g/g), along with its mono- and di-ethoxylates. Pesticides detected in dust from at least half the homes tested include permethrin, pentachlorophenol, piperonyl butoxide, chlordane, methoxychlor, propoxur, carbaryl, DDT, and folpet.

In this ongoing study, air and dust samples from 120 homes are being analyzed, and urine samples are being assayed for phthalate and pesticide metabolites. Self-reported data on product use in the home is also being collected from participants, and estrogenic activity in air samples is being evaluated by E-SCREEN bioassay. These sampling and analytical methods identify hormonally active chemicals and animal mammary carcinogens that are widespread in indoor environments, making them priorities for future research and regulatory evaluation. They also provide new exposure assessment tools for the study of hormonally mediated health outcomes.

Rudel, R.A.; Brody, J.G.; Spengler, J.D.; Vallarino, J.; Geno, P.W.; Sun, G.; Yau, A.; *Journal of the Air and Waste Management Association* **2001,** *51,* 499-513.
National Toxicology Program Center for the Evaluation of Risks to Human Reproduction. NTP-CERHR Expert Panel reports on seven phthalate esters, http://cerhr.niehs.nih.gov, 2000.
Centers for Disease Control and Prevention-National Center for Environmental Health. *National Report on Human Exposure to Environmental Chemicals,* www.cdc.gov/nceh/dls/report, 2001.

Ruthann Rudel, M.S.

Ruthann Rudel is a senior environmental toxicologist at Silent Spring Institute, with experience in toxicology, risk assessment and exposure assessment. One of Ms. Rudel's areas of research is the potential health effects of exposure to compounds that affect the endocrine system. She oversees the environmental assessment portion of the Cape Cod Breast Cancer and Environment study, a multi-disciplinary research effort funded by the Massachusetts Department of Public Health to study the elevated breast cancer incidence on Cape Cod. Ms. Rudel is the author of many articles in scientific journals, including a recent paper in *Journal of Air and Waste Management Association* about the identification of hormonally active agents and animal mammary carcinogens analyzed in commercial and residential air and dust samples. Ms. Rudel has also published articles in *Environmental Science and Technology* describing methods and results for testing groundwater for estrogenic phenols and a paper in *Environmental Health Perspectives* identifying issues in risk assessment for estrogenic chemicals. She has published journal articles and book chapters on regulatory toxicology, metals risk assessment, indoor-air risk assessment, and other subjects. She serves on the Science Advisory Board of the Massachusetts Toxics Use Reduction Institute. She has a B.A. in chemistry and neuroscience from Oberlin College, and an M.S. in environmental management and policy from Tufts University.

Silent Spring Institute, in Newton, MA, is a nonprofit scientific research organization dedicated to identifying the links between the environment and diseases that affect women, especially breast cancer.

☎ Tuesday, January 29, 2002 ☎

Risk Management Approaches

Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

4:00 PM *EPA's Risk Management Evaluation of EDCs*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

4:40 PM *Using Bioassays to Evaluate the Performance of Risk Management Techniques*
Carolyn Acheson, U.S. EPA, National Risk Management Research Laboratory

5:10 PM Adjourn for the day

EPA's RISK MANAGEMENT EVALUATION OF EDCs

Gregory D. Sayles
U.S. Environmental Protection Agency, Office of Research and Development
National Risk Management Research Laboratory, Cincinnati, OH 45268
(513) 569-7607, (513) 569-7105 fax
sayles.gregory@epa.gov

EPA's National Risk Management Research Laboratory (NRMRL) is developing a methodology to assess the current state of risk management of environmental problems. The findings from carrying out this methodology on a particular environmental challenge have been termed the Risk Management Evaluation (RME). In the last 2 to 3 years, pilot / demonstration RMEs were constructed for several environmental issues including endocrine disrupting chemicals (EDCs). Based on the experience gained in producing these pilot RMEs, a protocol to guide the writing of future RMEs is currently being finalized in NRMRL. The purpose of this presentation is to summarize the content of the RME for EDCs, which is in final draft form at this time.

The RME for EDCs presents in a succinct manner the current understanding of risk management of a short list of known or likely EDCs. The RME serves several purposes. First, the document identifies currently available risk management approaches that were developed for other purposes, but appear useful in managing the risk of EDCs. Second, the document indicates where new risk management approaches are needed. Thus, the RME will be useful (1) to inform risk managers such as regulators on what technical approaches are currently available for managing EDCs risk, (2) to educate the public about what risk management approaches are available now, (3) to motivate environmental consultants/engineers to review current skills or to develop new skills applicable to managing exposure to EDCs, and (4) to guide risk management researchers, such as NRMRL, in planning EDC risk management research programs.

Since health effects, exposure, risk assessment and risk management knowledge change with time, the RME must be updated regularly. Thus, we consider the RME as a living document to be updated as needed, approximately yearly, and downloadable from an EPA web site.

The presentation will discuss the specific content of version 1.0 of the RME for EDCs. This version will include discussion of the following known or likely EDCs: alkylphenol ethoxylates and related chemicals, natural, veterinary, and pharmaceutical steroid hormones, DDT and DDE, PCBs, and chlorinated dioxins and furans.

Gregory Sayles

Gregory Sayles is a chemical engineer with EPA=s National Risk Management Research Laboratory in Cincinnati, OH and leads the endocrine disrupting chemicals risk management research program. Dr. Sayles earned B.S., M.S., and Ph.D. degrees in chemical engineering from the California Institute of Technology, the University of California at Davis, and North Carolina State University, respectively. Dr. Sayles has worked for NRMRL for eleven years, conducting research mostly on bioremediation of contaminated soils and sediments.

USING BIOASSAYS TO EVALUATE THE PERFORMANCE OF RISK MANAGEMENT TECHNIQUES

Carolyn Acheson* and Gregory Sayles

U.S. Environmental Protection Agency, Land Remediation and Pollution Control Division, National Risk Management Research Laboratory
26 W. Martin Luther King Dr
Cincinnati, OH 45268
(513) 569-7190, (513) 569-7105 (fax)
acheson.carolyn@ epa.gov

Often, the performance of risk management techniques is evaluated by measuring the concentrations of the chemicals of concern before and after risk management efforts. However, using bioassays and chemical data provides a more robust understanding of the effectiveness of risk management strategies. For example, bioassay testing evaluates the aggregate effect of the environmental sample on the reporting organisms, and thus, includes aspects such as environmental matrix effects, sorption/desorption behavior, bioavailability, and chemical mixture interactions. As a result, bioassay testing can demonstrate changes in toxicity rather than inferring risk reduction from chemical concentrations. When bioassays are used to evaluate samples following risk management techniques, increased responses are observed in some cases. These increased responses may be due to incomplete treatment or toxicity introduced through process amendments. When these types of problems are identified through bioassay testing, risk management techniques can be altered to correct the problem. The combination of chemical and bioassay data has been helpful in evaluating risk reduction technologies treating soils contaminated with hazardous wastes. Due to the limited knowledge about the endocrine activity of various chemicals and their degradation products, bioassays are even more important in evaluating the performance of EDC risk management treatments.

Several EDC bioassays have been developed to identify EDCs or to characterize EDC health effects and exposure levels. To find an assay suitable for risk management projects, assays from the peer reviewed literature were evaluated based on: reported sensitivity, range of applications and chemicals studied, acceptance in the academic community, the details of the assay protocol, and the EDSTAC Tier I Screening Battery recommendations. Practical concerns such as cost, time, equipment and space needs, and personnel skills were also included in the evaluation process. Since most current risk management projects are concerned with estrogenic compounds, androgenic and thyroid assays were not considered at this time. Based on these criteria, the Yeast Estrogen Screening assay was selected as the first assay to be adapted for risk management projects in NRMRL.

In addition to discussing the benefits of including bioassay testing in evaluation of risk management effectiveness and the assay selection process, this presentation will discuss adapting a bioassay for NRMRL projects and a few hypothetical case studies illustrating the use of EDC bioassays to evaluate the effectiveness of risk management techniques.

Carolyn Acheson

Carolyn Acheson has been trained as a chemical engineer with emphasis in microbiology. She was awarded a Bachelor's of Chemical Engineering from the University of Delaware and a Ph.D. from Cornell University. She has worked at the U.S. EPA's National Risk Management Research Laboratory (NRMRL) since 1994. While at NRMRL, Dr. Acheson has studied the bioremediation of hazardous wastes particularly soils contaminated with polycyclic aromatic hydrocarbons. In many of these studies, remedial technology performance was evaluated using bioassays as well as the more traditional, chemical data. Based on her experience with soil toxicity, Dr. Acheson has recently begun a project to adapt EDC bioassays for use in evaluating remedial treatments for EDC contaminated matrices.

☎ Wednesday, January 30, 2002 ☎

Drinking Water Treatment

Moderator:	James Goodrich, U.S. EPA, National Risk Management Research Laboratory
8:35 AM	*Use of Granular Activated Carbon and Powdered Activated Carbon for the Removal of EDCs from Drinking Water: A User's Guide* John Cicmanec, U.S. EPA, National Risk Management Research Laboratory
9:05 AM	*Evaluation of Drinking Water Treatment Technologies for Removal of Endocrine Distrupting Compounds* Kathleen Schenck, U.S. EPA, National Risk Management Research Laboratory
9:20 AM	*Risk Management of Endocrine Disrupting Chemicals (EDCs) in Drinking Water* Frederick Pontius, Pontius Water Consulting
9:50 AM	Break

USE OF GRANULAR ACTIVATED CARBON AND POWDERED ACTIVATED CARBON FOR THE REMOVAL OF EDC'S FROM DRINKING WATER: A USER'S GUIDE

John L. Cicmanec, DVM, MS
U.S. EPA ORD NRMRL STD SAB
26 W. Martin Luther King, Jr. Drive
Cincinnati, OH 45268
(513) 569-7481, (513) 569-7585 fax
cicmanec.john@epa.gov

Recently, public concern has increased regarding industrial and environmental substances that may have adverse hormonal effects in human and wildlife populations. Although the list of potentially harmful substances is still being compiled and more sophisticated laboratory tests for detection of endocrine disrupting chemicals (EDCs) are being developed, an initial list of known EDCs has been made and an array of drinking water treatment processes has been evaluated for their ability to remove EDCs. Alkylphenols, bisphenol A, phthalates, polychlorinated biphenyls, dioxins, dibenzofurans as well as the pesticides methoxychlor, endosulfan, and DDT have been included in the initial list. In addition to the conventional water treatment processes of sedimentation, coagulation, and filtration, we have also considered the potential effects of granular activated carbon, powdered activated carbon, nanofiltration, reverse osmosis and air stripping for the removal of EDCs. Our findings indicate that granular activated carbon (GAC) and powdered activated carbon (PAC) are the most effective processes for the removal of the selected group of EDCs. The laboratory data that was used in the Freundlich equation for determining the efficiency of GAC for EDC removal will be presented. Additional analyses of the effectiveness for removing ethinylestradiol and melegesterol acetate through the use of GAC and PAC will also be presented.

John Cicmanec

Dr. Cicmanec is a graduate of the Kansas State University veterinary college and he has completed post-doctoral training at the University of Michigan Medical School. His clinical experience includes two years in large animal dairy practice and 15 years experience in biomedical research with non-human primates. This work involved infectious disease studies and part of this work included international scientific exchange programs with the Soviet Union, Bolivia and Peru. He has been at EPA for 16 years and his primary responsibilities have involved management of animal research colonies and noncancer risk assessment. He has been responsible for the risk assessments of PCBs and methylmercury and has participated in the review of 200 other chemical risk assessments. He was on the scientific team that wrote the Agency's Strategic Plan for Children's Health and his present research interests include endocrine disrupting chemicals and animal pathogens that are transmitted to humans.

EVALUATION OF DRINKING WATER TREATMENT TECHNOLOGIES FOR REMOVAL OF ENDOCRINE DISRUPTING COMPOUNDS

Kathleen Schenck [1*], Thomas Speth [1], Radha Krishnan[2], Barry Pepich[2], Steve Wendelken[2], Laura Rosenblum[2], Kent Mitchell[3], and David Warshawsky[3]

[1]U.S. EPA, National Risk Management Research Laboratory
26 West Martin Luther King Drive, Cincinnati, OH 45268
513-569-7947, fax: 513-569-7185, schenck.kathleen@epa.gov
[2] IT Corporation
11499 Chester Road, Cincinnati, OH 45246
513-569-7063, fax: 513-569-7707, krishnan.radha@epa.gov
[3] University of Cincinnati, College of Medicine, Department of Environmental Health
P.O. Box 670056, Cincinnati, OH 45267
513-558-5428, fax: 513-558-5561, mitchekm@email.uc.edu

Many of the chemicals identified as potential endocrine disrupting compounds (EDCs) may be present in surface or ground waters used as drinking water sources due to their introduction from domestic and industrial sewage treatment systems and wet-weather runoff. In order to decrease the risk of potential adverse health effects associated with the presence of EDCs in drinking water, two basic strategies exist. One is to protect source waters from contamination by EDCs. The other is to remove EDCs, which may be present in source waters, during the drinking water treatment process. This project addresses the latter approach by evaluating the removal of several EDCs by various drinking water treatment processes. The compounds to be evaluated are all steroid hormones: estradiol; estriol; ethynylestradiol; progesterone; testosterone and dihydrotestosterone. In the future, a group of nonylphenolic compounds will also be added. This project is divided into four parts. The first is the development of an analytical method to identify and quantify the analytes. The approach will include a solid phase extraction step followed by liquid chromatography/mass spectroscopy (LC/MS). The second is the application of a reporter gene assay, the MVLN assay, to evaluate the removal of estrogenic activity from the water samples. This assay uses a human breast cell line which has been transfected with the firefly luciferase reporter gene. This assay should detect the presence of compounds that have estrogenic activity, including those that may be missed analytically due to structural changes in the target compounds during treatment. Once the analytical and MVLN assays are in place, bench-scale evaluations of various drinking water treatment processes will be conducted. These will include conventional treatment, granular activated carbon, softening and nanofiltration. For each of these processes, pilot-scale evaluations may be conducted, if warranted.

Kathleen Schenck

Kathy Schenck is an environmental scientist with the Environmental Protection Agency, National Risk Management Research Laboratory. She has worked in the drinking water area for more than 20 years. She has a Master=s Degree in biology from the University of Cincinnati.

RISK MANAGEMENT OF ENDOCRINE DISRUPTING CHEMICALS (EDCs) IN DRINKING WATER

Frederick W. Pontius, P.E.
President
Pontius Water Consultants, Inc.
P.O. Box 150361
Lakewood, CO 80215-0361
(303) 986-9923, (303)716-7310 fax
fredp@pontiuswater.com

INTRODUCTION

Endocrine disrupting chemicals (EDCs) have emerged within the last decade as an important environmental issue. The implications for drinking water and water suppliers have only been seriously considered within the last several years. The U.S. Environmental Protection Agency (US EPA) Office of Ground Water and Drinking Water (OGWDW) is including EDCs and endocrine effects as it considers setting future drinking water regulations.

An April 2000 AWWA Research Foundation/Water Environment Research Foundation/ WaterRe-use Foundation workshop on Endocrine Disruptors and Pharmaceuticals in Drinking Water (Weyer and Riley, 2001) provided an opportunity for water suppliers to become informed about the science, potential health risks, regulation, and control of EDCs in drinking water. Water suppliers are considering how to minimize their customers risks to EDCs in drinking water.

DRINKING WATER REGULATION

Historically, U.S. EPA has considered endocrine effects in the regulation of drinking water contaminants when setting Maximum Contaminant Level Goals (MCLGs). Many contaminants that are known or suspected to be EDCs are already regulated in drinking water. Should new information regarding their endocrine effects become known, the Safe Drinking Water Act (SDWA) requires review and revision of existing regulations as needed at least every six years.

The endocrine effects of unregulated drinking water contaminants will be considered as part of the evaluation of contaminants listed on the Candidate Contaminant List (CCL). U.S. EPA will be making determinations for at least 5 contaminants on the CCL regarding whether regulation is needed, every 5 years, starting in 2001. Endocrine effects will be considered in these determinations.

A STRATEGY FOR WATER SUPPLIERS

Regardless of the regulatory actions taken by U.S. EPA, water suppliers must respond to customer concerns. As awareness of the potential effects of EDCs increases, customers naturally wonder whether EDCs are present in their drinking water. Many utilities have been actively addressing how they can minimize risk to customers from EDCs and communicate risks effectively with their customers. A simple risk management process can provide a framework for water suppliers to begin to address the complex issues associated with EDCs in drinking water.

REFERENCE

Weyer, P., and Riley, D. *Endocrine Disruptors And Pharmaceuticals in Drinking Water*. AWWA Research Foundation, Denver, Colo. (2001).

Frederick W. (Fred) Pontius

Fred Pontius is President of Pontius Water Consultants, Inc., in Lakewood, Colorado, a small company providing drinking water related professional engineering services to water utilities, consultants, industry, businesses, and regulatory agencies. He has 23+ years of experience in public water supply, including water quality control, design, research, and government affairs. He has prepared compliance and water quality control plans for large and small water systems, with consideration of endocrine disrupting chemicals (EDCs) and pharmaceuticals. Fred has written several books and over 150 technical articles on drinking water regulation, compliance, and water quality control, including EDCs. He is a frequent conference speaker, and has conducted training seminars for USEPA, the American Water Works Association, the National Park Service, and the Government Institutes. He is a registered professional engineer in Colorado and Ohio. In his spare time, Fred is a doctoral candidate in environmental engineering at the University of Colorado—Boulder, conducting research on membrane treatment.

☎ Wednesday, January 30, 2002 ☎

Concentrated Animal Feed Operations

Moderator: Laurel Staley, U.S. EPA, National Risk Management Research Laboratory

10:10 AM *Potential of Confined Animal Feed Operations (CAFOs) to Contribute Estrogens to the Environment*
Steven Hutchins, U.S. EPA, National Risk Management Research Laboratory

10:40 AM *Investigations of Sorption and Transport of Hormones and Animal Pharmaceuticals: Initial Laboratory Results*
Suresh Rao, Purdue University
Linda Lee, Purdue University

11:00 AM *Fate of the Endogenous Hormones 17ß-Estradiol and Testosterone in Composted Poultry Manure and their Sorption and Mobility in Loam Soil and Sand*
Heldur Hakk, USDA, Agricultural Research Service

11:30 AM Lunch

POTENTIAL OF CONFINED ANIMAL FEED OPERATIONS (CAFOS) TO CONTRIBUTE ESTROGENS TO THE ENVIRONMENT

Stephen R. Hutchins [1*], James N. Dumont[2], David M. Janz[2], G. Peter Breidenbach[3], Dennis D. Fine [3]
[1]U.S. EPA NRMRL, Subsurface Protection and Remediation Division
Robert S. Kerr Environmental Research Center, P.O. Box 1198, Ada, OK 74820
(580) 436-8563, (580) 436-8703 fax
hutchins.steve@epa.gov
[2]Department of Zoology, Oklahoma State University
430 Life Sciences West, Oklahoma State University, Stillwater, OK 74078
(405) 744-9683, (405) 744-7824fax
dumontj@okstate.edu
(405) 744-7593, (405)744-7824 fax
djanz@okstate.edu
[3]ManTech Environmental Services, Inc.
Robert S. Kerr Environmental Research Center. P.O. Box 1198, Ada, OK 74820
(580) 436-8668, (580) 436-8501 fax
breidenbach.peter@epa.gov
(580) 436-8669, (580) 436-8501 fax
fine.dennis@epa.gov

Abstract

Confined Animal Feed Operations (CAFOs) are a growing industry, with a trend towards fewer operations with higher concentrations of animals. Animals are fed and/or treated with many different types of pharmaceuticals, including antibiotics and hormones, which can end up in waste products. Some of these chemicals, in particular the natural and synthetic hormones, can exert endocrine-disrupting activity in the environment. The overall objective of this research is to evaluate whether animal wastes contain chemicals with EDC activity, and whether these chemicals are sufficiently persistent so as to pose a risk to receiving ground and surface waters.

Approach

There are many unknowns regarding the potential for CAFOs to contribute EDCs to the environment, due to the variety of CAFO operations, the diverse natures of potential EDCs themselves, and the different types of environmental receptors that could be affected. Hence, several projects to address these different issues are anticipated. Two projects have thus far been initiated, one to evaluate EDC activity from different types of CAFOs, and the other to measure levels of estrogens in swine waste effluents. These are described below.

EDC Activity in Different Types of CAFO Lagoons

This research is being conducted by Oklahoma State University under a cooperative agreement, and is designed to evaluate swine, dairy, and beef CAFO lagoons for EDC activity. Three analyses will be used to assess for the presence of EDCs. The first, the *Xenopus* Tail Resorption Assay (XTRA), measures the tail resorption rate prior to metamorphosis as a function of the presence of thyroid disrupters. The second measures plasma vitellogenin concentrations in male frogs using an enzyme-linked immunosorbent assay (ELISA) to detect the presence of estrogenic compounds. The third measures plasma testosterone and 17ß-estradiol concentrations as an indicator of alterations in reproductive endocrine homeostasis.

This research is ongoing. Preliminary results show that, although the swine effluent lagoon is quite toxic, none of the lagoons have exhibited significant EDC activity, at least based on these bioassays. It's quite possible that EDC activity is truly insignificant in CAFO lagoons. However, it may also be that these lagoons are insufficiently representative of large-scale commercial operations, or that there may be EDC effects under long-term exposure that are not detected with these bioassays.

Analysis of Estrogens in Swine Waste Effluents

Analysis of environmental samples for low levels of hormones is a rapidly-expanding field, and there have been many new developments in adapting current analytical techniques for these compounds. This work is being conducted by ManTech Environmental Services under a contract, and the primary objective is to optimize both ELISA and LC/MS/MS analytical procedures for analysis of complex wastewaters for estrogenic compounds, including 17ß-estradiol, estrone, estriol, and ethinyl estradiol. The goal is to use ELISA for screening of environmental samples for estrogenic activity, and then to confirm the presence of the individual estrogens in samples that test positive from ELISA. This work is being coordinated through SPRD so that different swine CAFO waste samples can be obtained, screened by ELISA, and then analyzed by LC/MS/MS to determine the concentrations of environmental estrogens.

This research is also ongoing. Preliminary results show that the analyses work well for ground water samples; detection limits are on the order of 0.05 ng/L for ELISA screening based on 17ß-estradiol, and 2 ng/L for estrogen separation and identification by LC/MS/MS. However, ELISA screening of swine lagoon effluents often yield 17ß-estradiol concentration estimates orders of magnitude higher than what can be confirmed by LC/MS/MS. HPLC clean-up of extracts reduces this interference, but does not eliminate it. LC/MS/MS analysis of several swine lagoon effluents shows that estrone is the most predominant estrogen.

Stephen R. Hutchins

Stephen R. Hutchins is a Research Environmental Scientist at EPA's National Risk Management Research Laboratory, Subsurface Protection and Remediation Division, located at the Robert S. Kerr Environmental Research Center in Ada, Oklahoma. Dr. Hutchins has a B.S. in Biology from the New Mexico Institute of Mining and Technology and a Ph.D. in Environmental Science from Rice University. His research interests are directed towards bioremediation of contaminated aquifers by indigenous subsurface bacteria, and his past work focused on laboratory and field evaluation of biorestoration of fuel-contaminated aquifers under anaerobic conditions. His current research project, funded under the NRMRL Laboratory Director's Special Grants Program, has been to evaluate the potential for ground water contamination from swine CAFOs.

INVESTIGATIONS OF SORPTION AND TRANSPORT OF HORMONES AND ANIMAL PHARMACEUTICALS: INITIAL LABORATORY RESULTS

Linda S. Lee[1], P. Suresh C. Rao[2]
[1]Agronomy Department, Purdue University
West Lafayette, IN 47907
(765) 494-8612
(765) 496-1107 fax
lslee@purdue.edu
[2]School of Civil Engineering & Agronomy Department, Purdue University
West Lafayette, IN 47907
765-496-6554
pscr@purdue.edu

With joint funding from U.S. EPA and the College of Agriculture, Purdue University, a series of laboratory studies has been initiated to evaluate sorption, transport and transformations of several compounds that have the potential to function as endocrine disruptors. The groups of test compounds investigated to date include: two hormones (estrodiol and testosterone), and three animal antibiotics (carbadox, tylosin, bacitracin) that are also used at sub-therapeutic levels as growth promoters in swine production. Initial project activities focused an extensive literature search to identify available environmental fate data and protocols for LC or GC analysis. Initial laboratory studies are focused on determining equilibrium adsorption isotherms using several Midwestern US soils and selected samples from a collection of soils and freshwater sediments that were used in past EPA investigations. These soil and sediments represent a wide range in sorbent characteristics, including pH, organic carbon content, clay content//type, CEC, geographic locations, etc. Batch isotherm data is being used to interpret the likely sorption mechanisms, and potential for leaching and retardation. Companion laboratory experiments are being conducted to evaluate transport and transformations under steady, saturated water flow conditions in columns packed with soils or sediment samples. The measured breakthrough curves are being interpreted using temporal moment analysis (to estimate mass conservation and retardation), and by fitting to existing transport models (to estimate retardation and non-equilibrium sorption parameters). Designs for a future field-scale test to examine retardation and degradation of the test compounds are also being developed based on preliminary tracer studies currently underway.

Linda S. Lee

Linda S. Lee is Professor of Environmental Chemistry & Soil Science in the Agronomy department at Purdue University. Since receiving a PhD from the University of Florida in 1991, she has been on the faculty at Purdue University. Her current research interests include remediation of soils contaminated with organics and metals, and basic research in environmental chemistry. She teaches courses in environmental organic chemistry and remediation science.

P. Suresh C. Rao

P. Suresh C. Rao is the Rieth Distinguished Professor of Environmental Engineering in the School of Civil Engineering, with a joint appointment in the Agronomy department, at Purdue University. Prior to arriving at Purdue two years ago, he was on the faculty for 25 years at the University of Florida. His current research interests include remediation science and engineering as well as watersheds and water quality. He teaches courses in subsurface hydrology and remediation.

FATE OF THE ENDOGENOUS HORMONES 17ß-ESTRADIOL AND TESTOSTERONE IN COMPOSTED POULTRY MANURE AND THEIR SORPTION AND MOBILITY IN LOAM SOIL AND SAND

Heldur Hakk[1*], Patricia Millner [2], and Gerald Larsen[1]
Colleen Pfaff [1], Barb Magelky[1], and Frank Casey[3]
[1] USDA-ARS Biosciences Research Lab, Fargo, ND
[2] USDA-ARS Soil Microbial Systems Lab, Beltsville, MD
[3] North Dakota State University, Dept. Soil Science, Fargo, ND
701-239-1293; 701-239-1430 fax
hakkh@fargo.ars.usda.gov

Relatively little attention has been paid to the fate and environmental impact of the potent hormones, 17ß-estradiol and testosterone, eliminated in animal and human waste. Laying chickens can excrete these hormones at 500 and 250 ng/g dry manure/day, respectively [1]. Furthermore, estradiol has a 100-fold greater affinity for the estrogen receptor than do man-made estrogenic substances of current environmental concern [2]. Land application of manure has a considerable potential to affect the environment adversely. Composting is known to effectively remediate soils contaminated with toxic organic compounds [3]. Thus, the present study was undertaken to quantitatively assess whether 17ß-estradiol and testosterone could be degraded during composting. Chicken layer manure was mixed with plant material to achieve a C:N ratio of 30:1, and place in 4 windrows (23 x 2 x 1.75 m). All windrows were turned weekly. Commercial enzyme immunoassay kits were used to quantitate the levels of 17ß-estradiol and testosterone in the aqueous extracts of samples. The results demonstrated that aerobic microorganisms degraded both hormones during the composting process. Composting resulted in a steady decline in extractable estradiol and testosterone levels. These data suggest that composting may be an environmentally friendly technology suitable for reducing the concentrations of these endogenous hormones at concentrated animal operation facilities.

Transport and fate experiments of [^{14}C] testosterone and 17ß-estradiol were conducted by individually applying each to columns packed with loam soil or sand. No radioactivity from either hormone was found in the effluent of the loam soil column. Combustion analysis of the soil sections showed that 80% and 96% of testosterone and estradiol, respectively, remained in the top five centimeters of soil. A nonequilibrium sorption convective-dispersive transport model was applied to the concentration profiles of the soil columns provided estimates of K_d values. Most of the testosterone and estradiol (87% and 85%, respectively) was eluted from the sand columns, respectively. Batch studies using the Glyndon silt-loam were conducted at 48, 96, and 178 h incubation times and Freudlich isotherms were fit to the observed data. For both hormones, the sorption isotherms were similar for all three incubation times and indicated that equilibration was reached by 48 h. The K_d values indicated that these chemicals readily sorbed to the soil.

[1] Shore, L.S., Gurevich, M., Shemesh, M., 1993, Bull. Environ. Contam. Toxicol., 51, 361-366.
[2] Jobling, S. and Sumpter, J.P., 1993, Aquatic Toxicology, 27, 261-272.
[3] US-EPA, 1998, Document EPA530-R-98-088, pp. 13-38.

Heldur Hakk

Heldur Hakk has been with the USDA/ARS/Biosciences Research Laboratory for 23 years, and is a Research Chemist in the Animal Metabolism-Agricultural Chemicals Research Unit. He obtained a PhD in Biochemistry from North Dakota State University in 1997. His areas of research include: mammalian metabolism of dioxins, PCBs, and brominated flame retardants, protein transport of chemicals in mammals, effect of exposure to xenobiotics on circulating hormones, applications of NMR to assess impact of foreign compounds to central metabolic pathways, and the fate of hormones in the environment.

☎ Wednesday, January 30, 2002 ☎

Wastewater Treatment

Moderator: Marc Mills, U.S. EPA, National Risk Management Research Laboratory

12:45 PM *Biological Fate of Estrogenic Compounds Associated with Sewage Treatment*: *A Review*
Gregory Sayles, U.S. EPA, National Risk Management Research Laboratory

1:15 PM *An Engineering Approach to Evaluate Estrogenic EDCs Fate During Wastewater Treatment*
Paul McCauley, U.S. EPA, National Risk Management Research Laboratory

1:30 PM Break

BIOLOGICAL FATE OF ESTROGENIC COMPOUNDS ASSOCIATED WITH SEWAGE TREATMENT

Gregory D. Sayles[1*], Tamara Marsh[2]
[1]U.S. Environmental Protection Agency, Office of Research and Development
National Risk Management Research Laboratory, Cincinnati, OH 45268
513-569-7607
513-569-7105 fax
sayles.gregory@epa.gov
[2]Elmhurst College, Dept. of Biology
190 Prospect Ave., Box 145
Elmhurst, IL 60126
(630) 617-3591
(630) 617-3735 fax
tamaram@elmhurst.edu

Considerable concern exists over the possibility that some man-made chemicals that mimic the effects of hormones may have adverse effects on reproduction of wildlife and humans. Like natural estrogens, these estrogenic compounds can bind to the estrogen receptor, thereby disrupting the normal function of the endocrine system. Reproductive toxicity has been attributed to prolonged exposure to environmentally relevant concentrations of estrogenic compounds. Two categories of endocrine disrupting chemicals are thought to be the greatest contributors of environmental contamination by estrogen mimicking chemicals. Naturally occurring hormones, such as 17?-estradiol and estrone, as well as chemically synthesized forms, including the main component in oral contraceptives, 17?-ethynylestradiol, by their very nature will make their way to sewage treatment plants (STPs). In addition, alkylphenol polyethoxylates (APEs), used in the production of surfactants, cleaning products, textiles, petroleum, pulp and paper, and pesticides can also mimic the effects of hormones to the endocrine system. Like the hormone class of chemicals, these compounds are present in sewage treatment plant influent. Endocrine disrupting chemicals, whether naturally occurring or chemically synthesized, may be transformed or partially degraded during the processes of aerobic and anaerobic sewage treatment. However, these chemicals or their derivatives are accumulating in ecosystems impacted with sewage treatment effluent, indicating that they are not being adequately degraded in the sewage treatment plant. Alkylphenol polyethoxylates have been shown to lose portions of the polyethoxylate chain during sewage treatment, however, the most prevalent resultant compound, nonylphenol (NP) is known to accumulate and cause toxicity in aquatic organisms even at low concentrations. Thus, the degradation product poses more risk to the environment than the parent compound. While less is known about the fate of the hormone compounds, evidence suggests that these compounds are also accumulating in the environment and causing reproductive failure in a variety of wildlife. This presentation will examine the current state of knowledge concerning the microbial degradation of these categories of endocrine disrupting chemicals, specifically their fate at the sewage treatment level as well as long-term fate once they are released into the environment.

References

Hesselsøe, M., Jensen, D., Skals, K., Olesen, T., Moldrup, P., Roslev, P., Mortensen, G.K., and Henriksen, K. 2001. Degradation of 4-Nonylphenol in Homogeneous and Nonhomogeneous mixtures of soil and sewage sludge. Environ. Sci. & Technol. (In Press).

Ternes, T.A., Kreckel, P., and Mueller, J. 1999. Behaviour and occurrence of estrogens in municipal sewage treatment plants – II. Aerobic batch experiments with activated sludge. Sci. Total Environ. 225: 91-99.

Gregory Sayles

Gregory Sayles is a chemical engineer with EPA=s National Risk Management Research Laboratory in Cincinnati, OH and leads the endocrine disrupting chemicals risk management research program. Dr. Sayles earned B.S., M.S., and Ph.D. degrees in chemical engineering from the California Institute of Technology, the University of California at Davis, and North Carolina State University, respectively. Dr. Sayles has worked for NRMRL for eleven years, conducting research mostly on bioremediation of contaminated soils and sediments.

AN ENGINEERING APPROACH TO EVALUATE OF ESTROGENIC EDCS FATE DURING WASTEWATER TREATMENT

Paul T. McCauley
U.S. EPA, National Risk Management Research Laboratory
26 West Martin Luther King Drive
Cincinnati, OH 45268
(513) 569-7444
(513) 569-7105 fax
McCauley.Paul@EPA.gov

Evidence is accumulating that environmental EDCs are affecting wildlife populations. Environmental EDCs appear to have a range of effects. Some studies report unusual gene induction patterns in male fish, while others have indicated that EDCs may result in hermaphrodism in native fish populations.

One source of environmental EDCs are POTWs. Desbrow et al. 1998, reported POTWs discharged several estrogenic steroids including: estrone, 1 to 50 ng/L; 17 ß-estradiol, 2 to 50 ng/L; and 17 a-ethynylestradiol, 0 to 7 ng/L. At these levels, significant induction of vitellogenin was observed in several fish species. Others have speculated that alkylphenols, three ethoxylates and metabolites may have estrogenic effects.

We will be investigating the effectiveness of wastewater treatment plants and various steps in wastewater treatment at removing estrogenic EDCs. Two conventional activated sludge pilot plants, one employing anaerobic sludge digestion and the other aerobic sludge digestion are currently being built. The pilot plants are to be identical in all respects except for the method of sludge digestion. Because many POTWs today have stringent ammonia nitrogen discharge standards, the pilot plants are designed to nitrify. In addition to facilitating EDC analytical methods development through the provision of liquid stream samples of known EDC compound concentrations, these parallel flow regimes will provide comparative information on the two most common in-plant means of stabilizing and reducing the mass of waste sludges used in municipal wastewater treatment plants. The pilot plant trains will consist of the following unit processes:

$ Primary clarifier (both trains)
$ Aeration tank (both trains)
$ Secondary clarifier (both trains)
$ Single-stage, high-rate anaerobic sludge digester (anaerobic digestion train only)
$ Aerobic sludge digester (aerobic digestion train only)
$ Sludge dewatering unit (both trains)

Chemical methods including solid phase extraction and GC/MS will be used to measure steroids and smaller ethoxylate chain nonyl phenols and normal phase HPLC will be employed to analyze larger ethoxylate chain nonyl phenols. Biological methods including mRNA (vitellogenin) induction analysis in Fathead Minnow and a recombinant yeast assay will be used. These methods will be employed to measure the removal rates of the various estrogenic EDC at several points in the pilot plant wastewaster treatment process (including both liquid and solid waste streams). When steady state results are achieved, the pilot plants will be adjusted to maximize estrogenic EDC removal. After optimization is complete these analysis will be taken to the field at operating wastewater treatment plants. We will evaluate these plants for Estrogenic EDC removal with attention to optimization of the removal process. The final output of this research effort will be to make final recommendations for improved estrogenic EDC removal in publicly owned wastewater treatment plant.

Desbrow et al. (1998) Environmental Science and Technology 32 (11):1549-1556

Paul T. McCauley

Dr. McCauley is a chemist with the U.S. EPA National Risk Management Laboratory (NRMRL_ Land Remediation and Pollution Control Division Treatment and Destruction Branch. He has a Ph.D. from Ohio State University, College of Medicine Department of Pharmacology. He has worked predominantly in bioslurry and bioventing vadose zone remediaiton. Dr. McCauley has published papers in metals toxicity (pb, Ba, A, and orano-tins), kinetics, central nervous system, endocrine, and general toxicology including: cardiovascular and hepatic toxicology.

☎ Wednesday, January 30, 2002 ☎

Other EDC Risk Management Challenges

Moderator: Andy Avel, U.S. EPA, National Risk Management Research Laboratory

1:45 PM *Endocrine Disruptors from Combustion and Vehicular Emissions: Identification and Source Nomination*
Brian Gullett, U.S. EPA, National Risk Management Research Laboratory

2:00 PM *Natural Recovery of PCB-Contaminated Sediments at the Sangamo-Weston / Twelve Mile Creek / Lake Hartwell Superfund Site*
Richard Brenner, U.S. EPA, National Risk Management Research Laboratory
James Lazorchak, U.S. EPA, National Exposure Research Laboratory

2:45 PM *Program for the Identification and Replacement of Endocrine Disrupting Chemicals*
Douglas Young, U.S. EPA, National Risk Management Research Laboratory

3:00 PM Adjourn Workshop

ENDOCRINE DISRUPTORS FROM COMBUSTION AND VEHICULAR EMISSIONS: IDENTIFICATION AND SOURCE NOMINATION

Brian Gullett[1*], Jeff Ryan[1], Paul Lemieux[1], Carolyn Acheson[2], Michael DeVito[3], James Rabinowitz[3], Sukh Sidhu[4], Richard Striebich[4], Joy Klosterman[4]

U.S. Environmental Protection Agency
[1]National Risk Management Research Laboratory, Air Pollution Prevention and Control Division (MD-65), Research Triangle Park, NC 27711
[2]National Risk Management Research Laboratory, Cincinnati, OH 45268
[3]National Health and Environmental Effects Research Laboratory, Toxicology Division, Research Triangle Park, NC 27711
[4]University of Dayton Research Institute, 300 College Park, Dayton, OH 45469-0001
(919) 541-1534, (919) 541-0554 fax
gullett.brian@epa.gov

Exhaust samples from combustion and vehicular sources are being analyzed to provide initial identification of endocrine disrupting chemicals (EDCs). The intent of this screening effort is to provide discerning evidence for nominating sources for further EDC characterization. Conventional sampling, advanced analytical methods, and bioassays are being used to provide initial characterization of these samples for their compound identity and EDC activity. Our intent is to sample and chemically characterize multiple combustion sources, consistent with the likelihood that combustion source EDC exposure is not linked to any one source. For example, since body burdens of polychlorinated dibenzodioxin/dibenzofuran do not appear to be elevated near traditionally suspected sources (e.g., waste incinerators), it appears that exposure sources of dioxin are ubiquitous. Sample fractionation will be coupled with chemical characterization, structure activity analyses, and bioassay testing to nominate and identify potential EDC compounds in combustion emissions. Our ability to provide this early source-specific EDC identification and characterization of combustion sources will be a parallel activity with more extensive health risk analysis and exposure assessment. In this manner, appropriate prioritization of EDC management options can be implemented prior to definitive health effects conclusions.

A variety of combustion sources and fuel types will be opportunistically sampled, including diesel fuels, fuel oil, biomass, natural gas, coal, refuse fuels, municipal and household wastes, and hazardous waste. HPLC extract fractionation and estrogenicity testing using the Vitellogenin mRNA Assay will be done on the samples with EPA/NEHERL and NRMRL/Ci. Multidimensional gas chromatography (MDGC) by UDRI will characterize the sample unknowns and identify obscured peaks.

Some samples have been analyzed via coventional GC- and MDGC-MS analysis and found to be predictably complex. Currently we are investigating the analysis of individual compounds which have structures similar to alkyl phenols (such as nonylphenol, a suspected endocrine disruptor) as well as polycyclic aromatic hydrocarbons (metabolites of which can interact with the estrogen receptor). Many compounds have been found that would have been difficult to detect in conventional GC-MS because of coelution. For example, a methoxy alkyl phenol was found to coelute with biphenyl under conventional conditions but was well separated using MDGC.

Brian Gullett

Brian Gullett works in the area of trace combustion by-product formation, mainly chlorinated dibenzodioxins and dibenzofurans (CDD/F), as well as fundamentals of mercury (Hg) sorption. His current work includes development of the application of Jet Resonance-Enhanced MultiPhoton Ionization (REMPI) toward use as a trace toxics (including CDD/F) detector, determining the reaction kinetics of CDD/F formation, and discernment of the kinetics and site-specific functional groups of Hg reaction with sorbents. He has written over 125 papers; lectured in the US, Germany, Australia, Sweden, and Korea; and served as conference/session chairs for international symposia on CDD/F formation. He has twice been the recipient of the EPA's Federal Engineer of the Year award and holds seven EPA Scientific and Technological Achievement Awards. Brian is Air Pollution Editor for *Environmental Engineering Science* as well as a reviewer for a number of journals. In '95-'96 his work included a one year assignment to the US Naval Surface Warfare Center where he worked on shipboard incineration systems. He also serves as the Compliance pillar Co-Chair of the Department of Defense's Strategic Environmental Research and Development Program (SERDP). He has earned an A.B., an M.S. and Ph.D. in Environmental Engineering and, more recently, a Master's of Engineering Management, all from Duke University. Needless to say, he is a basketball fan.

NATURAL RECOVERY OF PCB-CONTAMINATED SEDIMENTS AT THE SANGAMO-WESTON/TWELVE MILE CREEK/LAKE HARTWELL SUPERFUND SITE

Richard C. Brenner[1*], James M. Lazorchak[2*], and Victor S. Magar[3]

[1] U.S. EPA/NRMRL, 26 W. Martin Luther King Drive, Cincinnati, OH 45268
(513) 569-7657, (513) 569-7105 fax
brenner.richard@epa.gov
[2] U.S. EPA/NERL, 26 W. Martin Luther King Drive, Cincinnati, OH 45268
(513) 569-7076, (513) 569-7609 fax
lazorchak.jim@epa.gov
[3] Battelle, 505 King Avenue, Columbus, OH 43201
(614) 424-4604, (614) 424-3667
magarv@battelle.org

Lake Hartwell is an artificial lake located in the northwest corner of South Carolina along the Georgia state line. It was created between 1955 and 1963 when the US Army Corps of Engineers constructed Hartwell Dam on the Upper Savannah River. It is the second largest lake in South Carolina by volume and the third largest by surface area (56,000 acres).

The Sangamo-Weston plant was used for capacitor manufacturing from 1955 to 1978. The plant used a variety of dielectric fluids in its manufacturing processes, including fluids containing PCBs. Waste disposal practices at the site resulted in the cumulative discharge over the 23 years of plant operation of an estimated 400,000 lbs of PCBs into Town Creek, a tributary of Twelve Mile Creek, which in turn is a tributary of Lake Hartwell. Source control implemented by EPA's Region 4 in the 1990s has successfully stopped the flow of PCBs into Lake Hartwell and its tributaries. With the completion of source control, the PCB-contaminated Lake Hartwell ecosystem became a viable candidate for natural recovery. Natural recovery was selected by the Region as the remedy of choice for this site, as specified in its Record of Decision in 1994.

A field evaluation study was conducted in 2000 using a quantitative approach initiated by EPA/NRMRL under contract to Battelle in cooperation with Region 4. The study characterized the contaminated area and estimated the degree of recovery achieved in contaminated Lake Hartwell sediments to date. This study has continued into 2001 with a second sediment sampling effort by EPA/NRMRL and Battelle, combined with a fish toxicology evaluation by EPA/NERL. For both the 2000 and 2001 sampling efforts, sediment cores (▾60 to ▾100 cm long x 5 cm diameter) were collected from predetermined transect locations. The cores were divided into 5-cm segments. Each segment was analyzed for PCBs to determine concentration profiles and ^{210}Pb and ^{137}Cs to age date the sediment deposits. A total of 107 PCB congeners were quantified to characterize the source of PCBs and the extent of dechlorination with sediment depth.

The data indicated that the net deposition of clean sediment has resulted in substantial burial of contaminated sediment. In the six downgradient cores not affected by release of sediment from upstream impoundments, the times to achieve three successively more stringent cleanup goals of 1.0, 0.4, and 0.05 mg/kg t-PCBs were estimated at 1 to 3, 2 to 10, and 8 to 30 years, respectively, depending on clean sediment accumulation rates at each transect. PCB contamination at depth was associated primarily with silt and silt/clay sediment layers. Little to no contamination was found in sand layers. PCB congener compositions became increasingly dominated by lower chlorinated congeners with sediment depth and corresponding age, suggesting that dechorination is occurring to some extent in the deeper and older sediments. This hypothesis was supported by a relative accumulation of *ortho* and loss of *meta* and *para* chlorines in the PCBs analyzed in these sediments. These minor changes in the degree of chlorination do not in any way imply a reduction in risk from PCB contamination in these sediments.

Water and sediment samples were also collected from Lake Hartwell and a background site to determine if PCBs in the sediments were bioavailable (by measuring fathead minnow liver cytochrome P450IA1 gene expression) and/or if they were estrogenic (by measuring male fathead minnow liver vitellogenin gene expression). Four water column samples (one each from the background site and three transect locations) were tested on May 10, 2001 using 11 - 13 month old adult male fathead minnows and 42-hr old fry. Fry were exposed to Lake Hartwell water for 48 hr and the adult males for 24 hr. In addition to the Lake Hartwell samples, two positive controls and two laboratory water controls were also tested. The positive controls were 50/50 mixtures of Arochlors 1242 and 1258 at 10 and 100 ng/L. Nine sediments samples were tested with fathead minnows. Two moderately hard laboratory water controls (without DMSO and with DMSO at 3.74 ▾g/L) and one positive control (50/50 mixture of Arochlors 1242 and 1258 at 10 ng/L) were also tested. Adult fish livers were necropsied after exposures, and RNA was isolated to determine if the expression of P450IA1 and/or vitellogenin genes was increased. Fry were collected after each exposure, and RNA was extracted and selectively amplified by PCR to also determine if these genes were being expressed in embryos exposed to sediments. Preliminary results of this work will be presented at the Workshop.

Richard C. Brenner

Richard C. Brenner is an Environmental Engineer at the U.S. EPA National Risk Management Research Laboratory (NRMRL) in Cincinnati, OH. He as a MS in Environmental Engineering and a BS in civil Engineering from the University of Cincinnati. Mr. Brenner has many research interests including: bioremediation and natural recovery of contaminated sediments, biotreatment and chemical oxidation/biotreatment of contaminated soils, and fate of EDCs during wastewater treatment. He has been the senior author or co-author on over 25 peer-reviewed journal articles. Mr. Brenner has worked on over 35 conference proceedings articles and presentations.

James M. Lazorchak

Dr. James Lazorchak is a research Aquatic Ecologist and toxicologist at the US EPA Office of Research and Development's Ecosystem Research Branch in Cincinnati, OH. As a Senior Ecotoxicologist and manager of an AAALAC Certified Aquatic Research facility he is responsible for exposure toxicity and molecular methods development and research. Dr. Lazorchak's latest research is in the area of real-time biological monitoring using clams, daphnia, algae and fish to detect episodic and long-term exposures to contaminants. He is also conducting research on the use of gene expression in fathead minnows to detect estrogenic compounds, metals and chlorinated persistent chemicals. He is the indicator lead for research and assessment methods for fish contamination, water and sediment toxicity. Dr. Lazorchak is also the co-lead for zooplankton and macroinvertebrates. Dr. Lazorchak is responsible for exposure design for genetic variation testing with fathead minnows and amphipods. He has a Doctor of Philosophy in Ecotoxicology from the University of Texas at Dallas. Dr. Lazorchak has a MS in Environmental Sciences also from the University of Texas at Dallas, as well as a MS in Aquatic Ecology from Wright State University. He has a BS in Zoology from Southeast Missouri State University. Dr. Lazorchak has been published in numerous journals and US EPA publications including: Environmental Toxicology and Chemistry, and Aquatic Toxicology.

PROGRAM FOR THE IDENTIFICATION AND REPLACEMENT OF ENDOCRINE DISRUPTING CHEMICALS

Douglas Young Douglas Young
U.S. Environmental Protection Agency
Office of Research and Development
National Risk Management Research Laboratory
26 W. Martin Luther King Dr., MS-466
Cincinnati, OH 45268
(513) 569-7624
(513) 569-7111 fax
young.douglas@epa.gov

A computer software program is being developed to aid in the identification and replacement of endocrine disrupting chemicals (EDC). This program will be comprised of two distinct areas of research: identification of potential EDC and suggestions for replacing those potential EDC. This identification portion of the program will be accomplished by constructing virtual 3-D representations of potential EDC and then comparing these representations to a library of known EDC and to a library of chemicals that have been identified not to be an EDC. These libraries will most likely focus around the estrogen receptor sites since this is where a vast majority of the data is. This identification portion of the research will be conducted external to the US EPA. The second phase of the program will focus on suggesting replacements. This portion of the program will suggest a series of possible replacements for potential EDC. The suggested replacements will be a chemical or a mixture of chemicals that will best match the appropriate chemical and physical properties of the EDC. The portion of the program will be configured similar to the software program PARIS II (Program for Assisting the Replacement of Industrial Solvents), which was developed within the US EPA. This project to identify and replace EDC is scheduled to begin in the Fall of 2001. This presentation will introduce a detailed plan for this project.

Douglas Young

Dr. Young is a chemical engineer working at the U.S. Environmental Protection Agency's National Risk Management Research Laboratory in Cincinnati, OH. There he leads the Simulation and Process Design Team. Dr. Young's research is in the areas of environmental impact assessment as it pertains to the chemical processing industry and the estimation of acute toxicity measurements by structural relationships. Dr. Young was instrumental in the development and commercialization of the generalized Waste Reduction (WAR) algorithm. Dr. Young received his Ph.D. from the University of Arizona where his dissertation focused on the bioremediation of high-energy explosive waste generated at the Los Alamos National Laboratory. He received his M.S. from The University of Notre Dame and his B.S. from The University of Michigan. All of Dr. Young's degrees are in chemical engineering.

Using Bioassays to Evaluate the Performance of Risk Management Techniques

Carolyn Acheson, M. Christina Brinkman, and Gregory Sayles

Common Risk Management Assumptions

- Risk - characterized by contaminants - Ignores
  - Incomplete removal or side products
  - Co-Contaminants
  - Matrix Effects
- Treatment reduces toxicity - Ignores
  - Process Amendments
  - Other reactions
  - Matrix Changes

Case Study 1

- Remediation of PCB Contaminated Soil by Solvent Extraction
- Principals: Mark Meckes, John Meier, and Lina Chang
- More information - Meier, et al. 1997. Environmental Toxicology and Chemistry, p. 928 - 938.

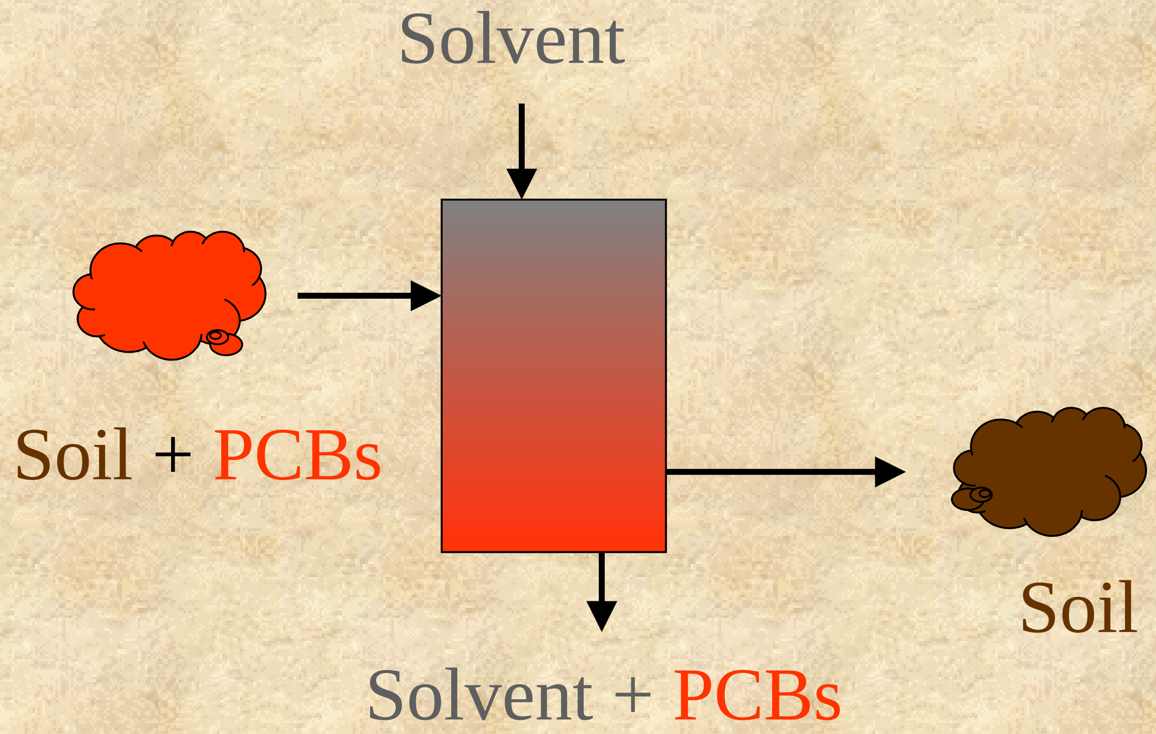
Case Study 1 - Chemical Analysis

- PCBs
- VOCs
- SVOCs
- Metals by TCLP

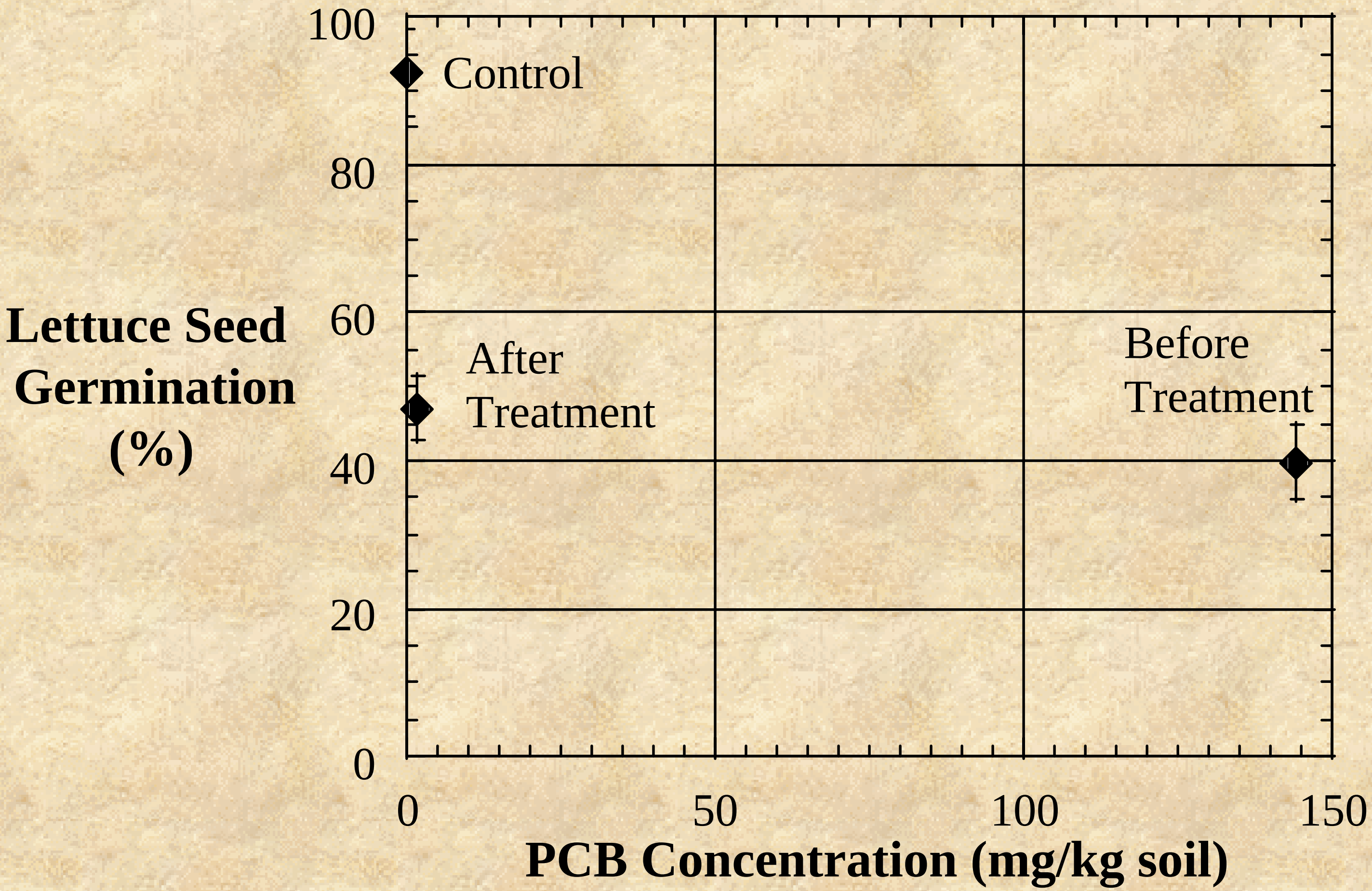
Case Study 1 - Bioassays

Bioassay	Organism	Exposure Matrix	Exposure Period	Endpoint
Earthworm Survival	E. fetida L. terrestris	soil	14 days	Survival
Seed Germination	Oats and Lettuce	soil	5 days	Survival
Earthworm Reproduction	E. fetida	soil	3 weeks	Survival, body mass, number of cocoons, cocoon hatchability
Root Elongation	Oats and Lettuce	soil	5 days	Growth
Allium Mitotic Aberrations	Allium	water extract	24 hour	Mitotic index, chromosomal abnormalities

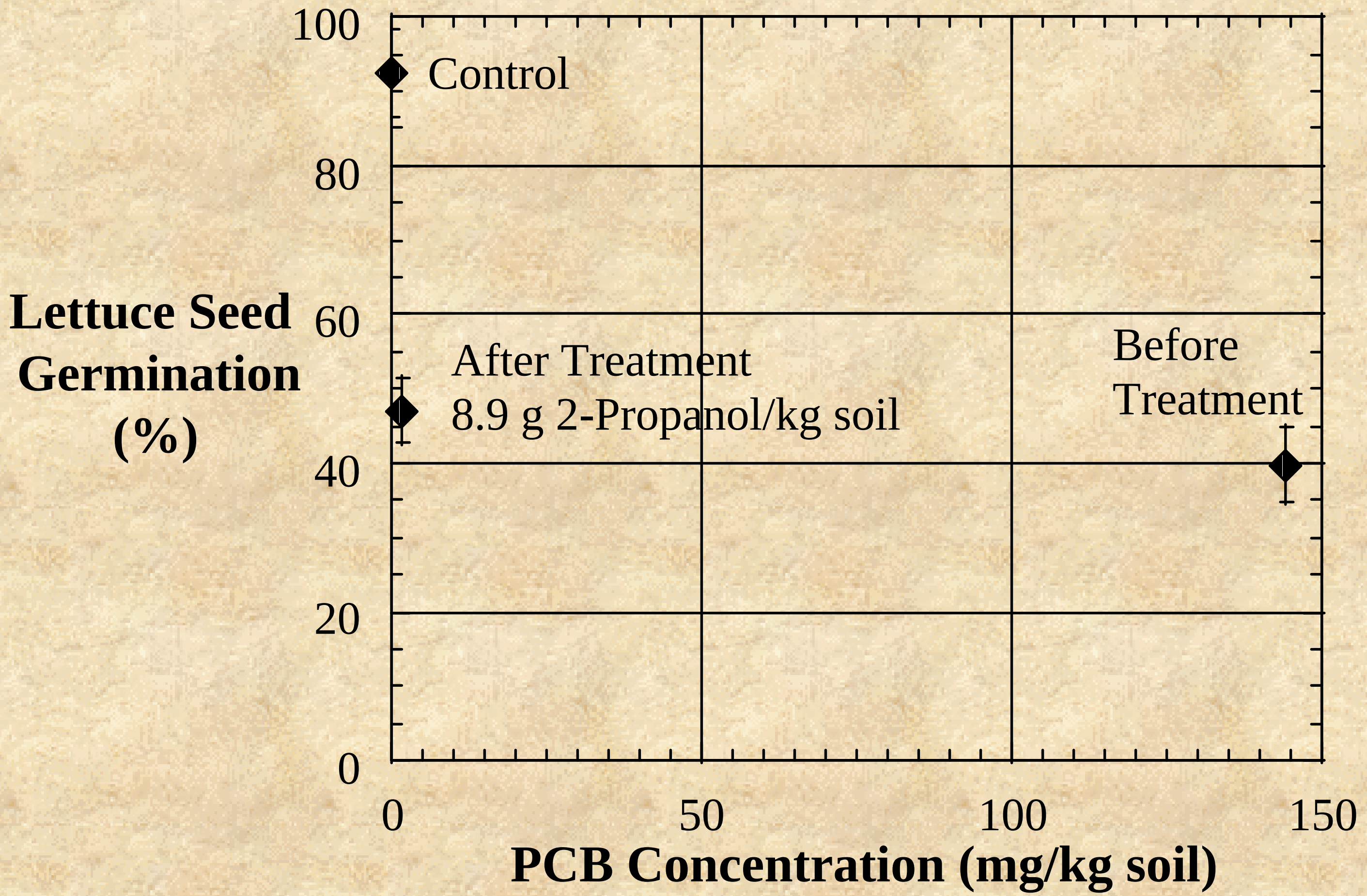
Case Study 1 - Solvent Extraction



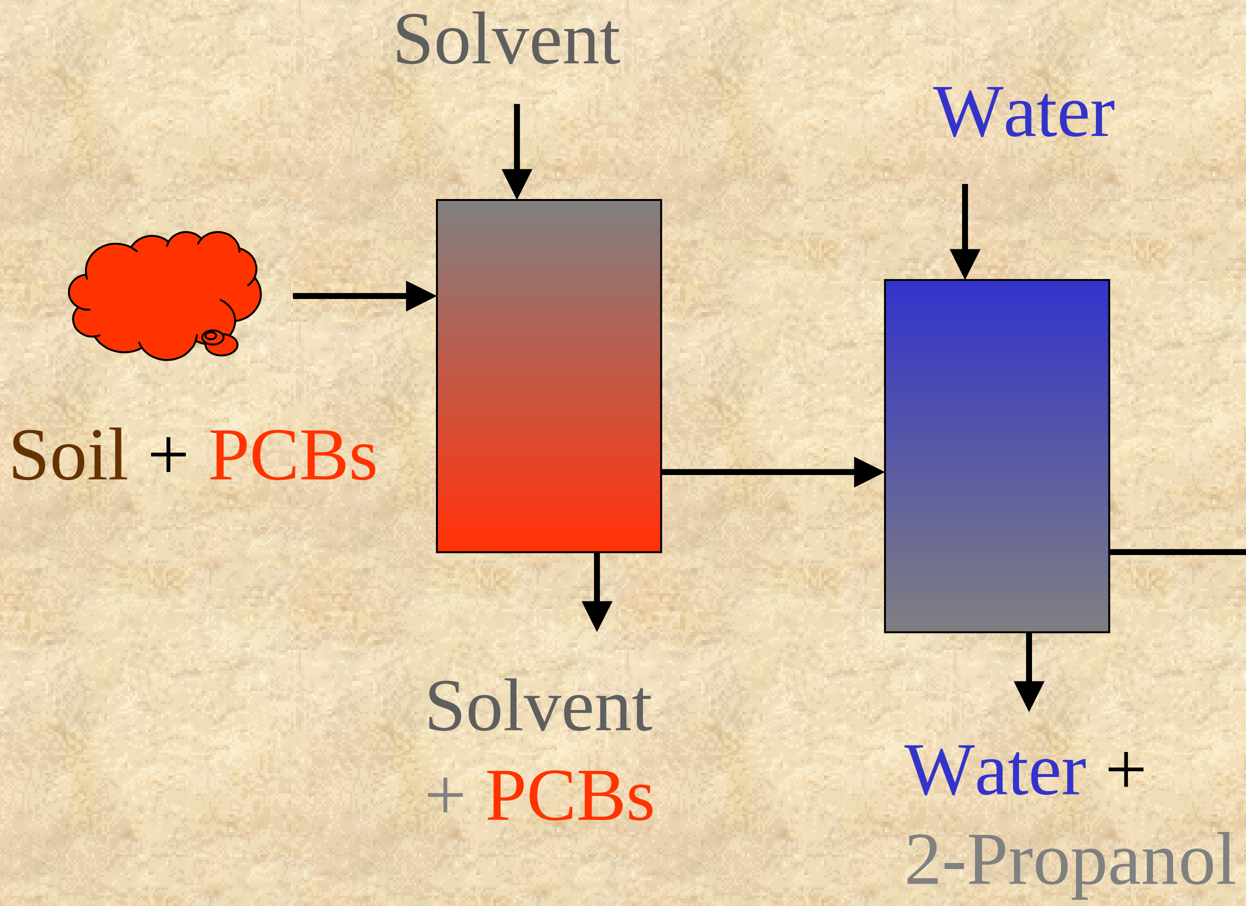
Case Study 1 - Results



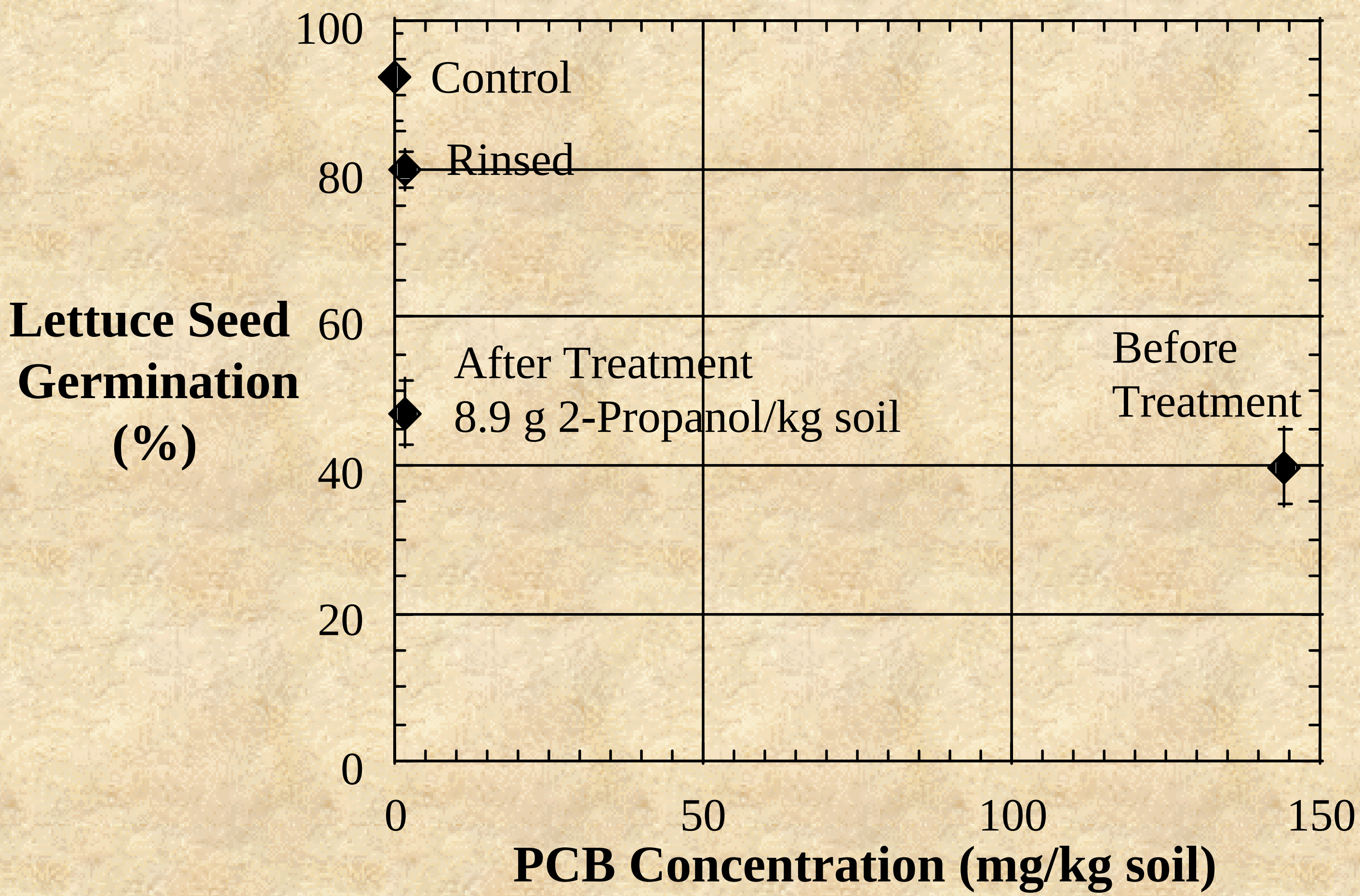
Case Study 1 - Results



Case Study 1 - Better RM



Case Study 1 - Results 2



Case Study 1 - Summary

- Solvent extraction removed PCBs from soil
- Process residues were as toxic as PCBs
- Better RM - Add rinse step
  - Reduce PCB concentration
  - Reduce toxicity

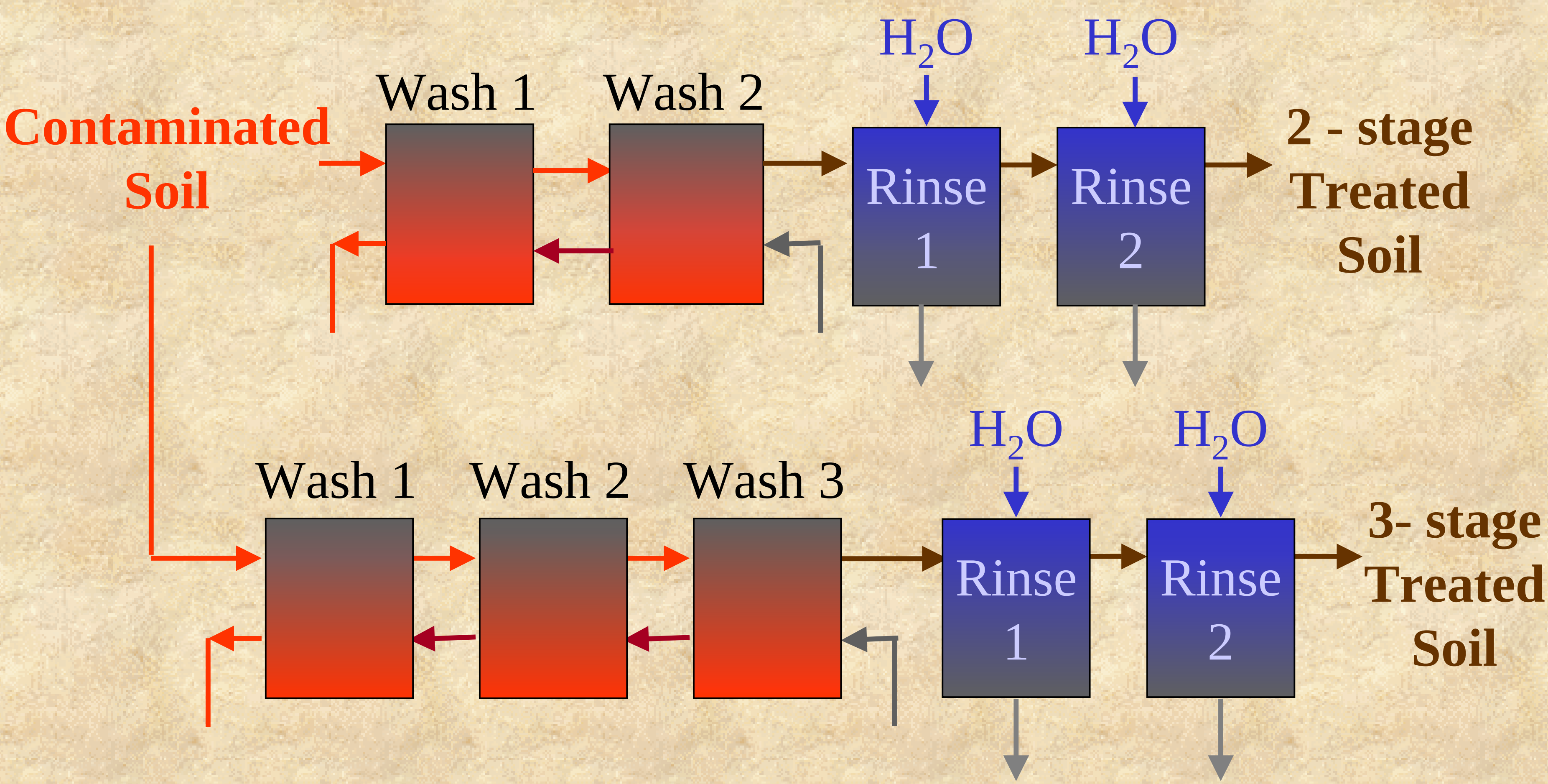
Case Study 2

- Remediation of soil contaminated with wood treating wastes by Soil Washing
  - Fluid - Ethanol-water mixture
  - Question - 2 or 3 Soil Washing stages?
- Principals
  - Soil Washing - Richard Brenner, Makram Suidan, George Sorial, Amid Khodadoust, Karen Koran, and Gregory Wilson
  - Ecotoxicity Evaluation - Carolyn Acheson, Jennifer Mansfield , Yonggui Shan, and Margaret Kupferle

Case Study 2

- Chemical Analysis
  - PCP
  - Hydrocarbons - alkanes and PAHs
- Bioassays
  - Earthworm Survival
  - Seed Germination and Root Elongation in Lettuce and Oats

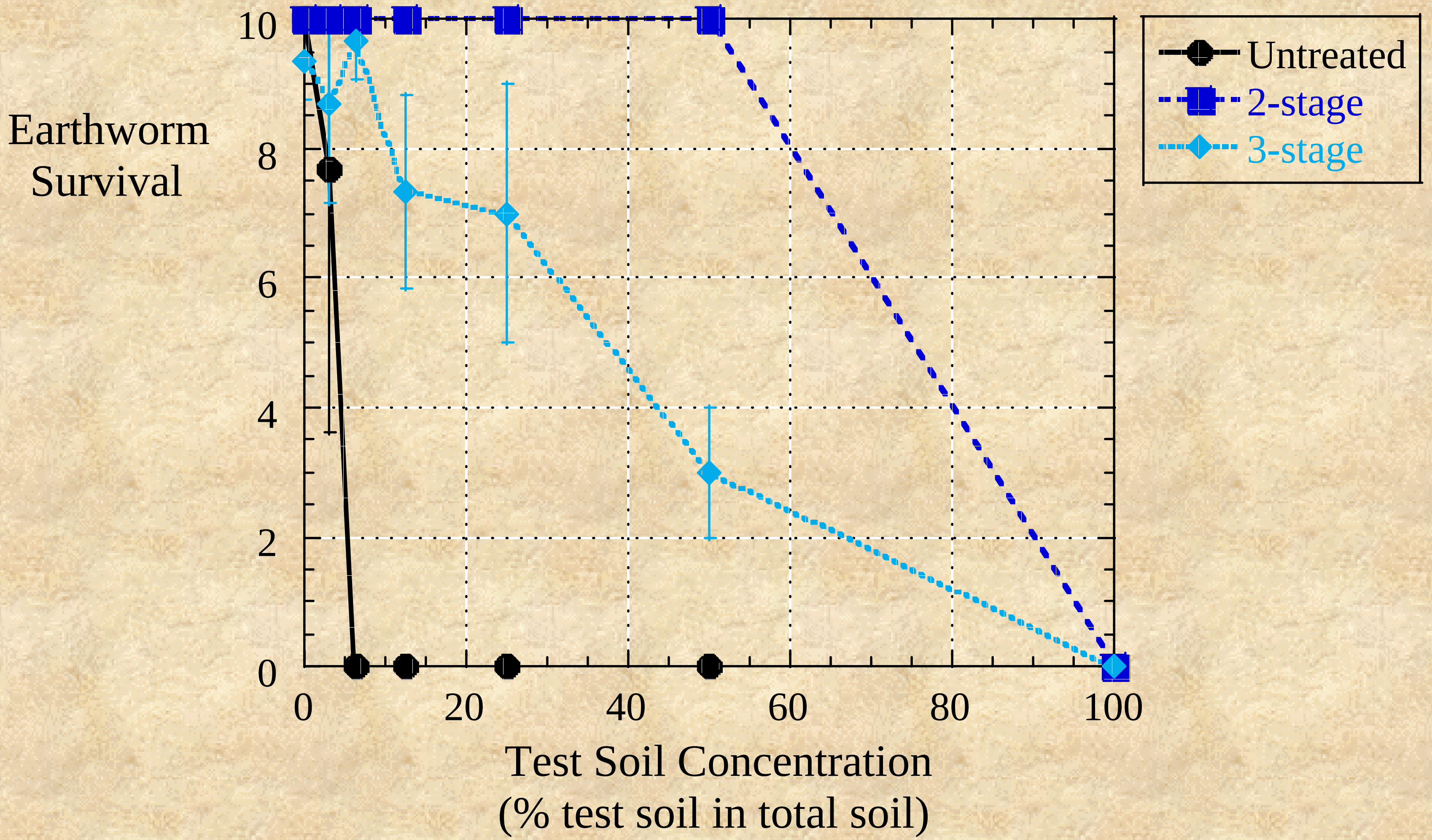
Case Study 2 - Soil Washing



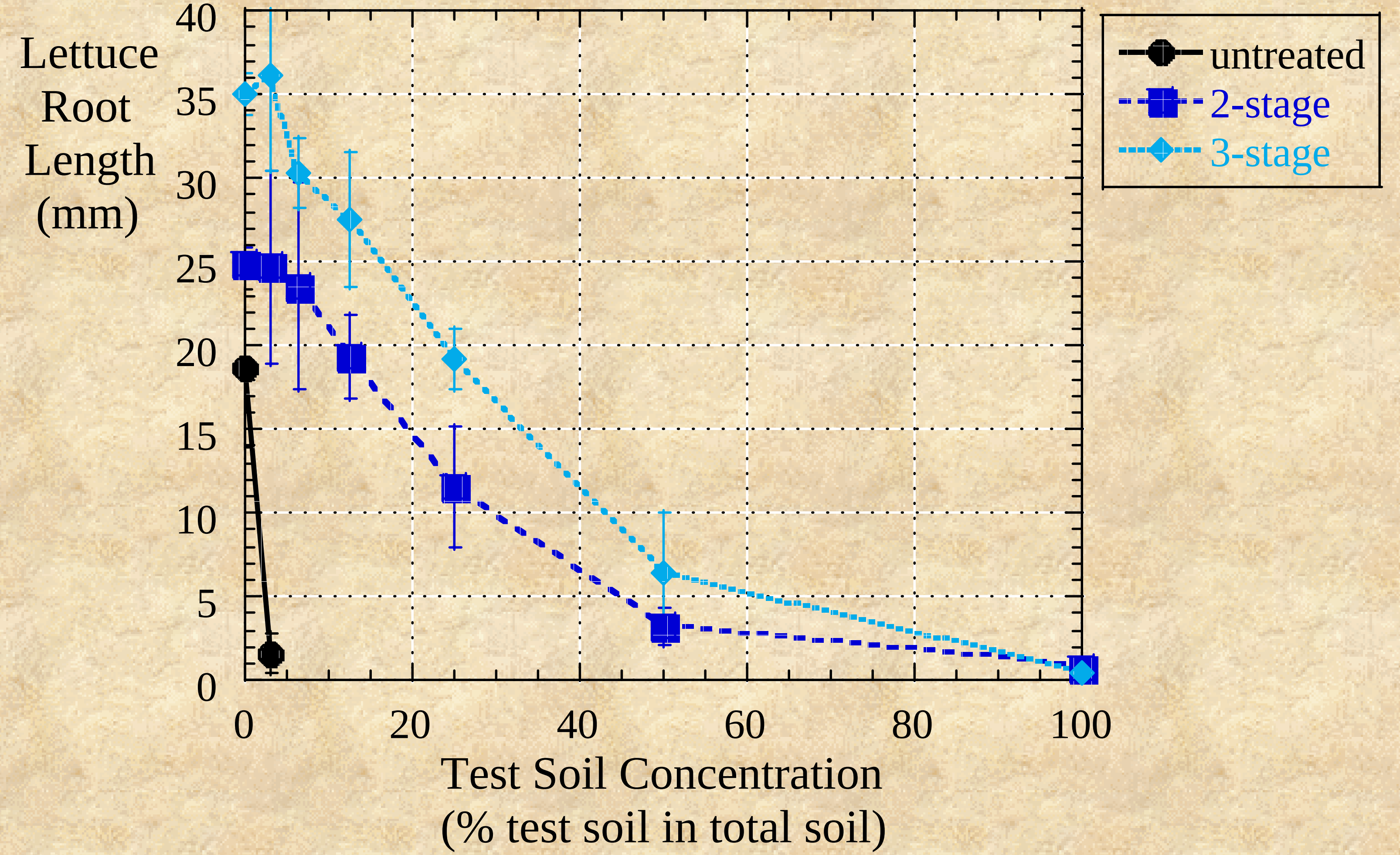
Case Study 2 - Results

Chemical	**Concentration (mg/kg dry soil)**		
	Untreated	2-stage	3-stage
PCP	950 ± 51	31± 1	9 ± 1
Alkanes	1761 ± 46	130 ± 17	59 ± 12
PAHs	494 ± 20	12 ± 2	<3

Case Study 2 - Results



Case Study 2 - Results



Case Study 2 - Results

Bioassay	Untreated	2-stage	3-stage
LC 50 (% test soil in total soil)			
Earthworm Survival	4.0	70.7	35.0 (26.3, 43.4)
Lettuce Seed Germination	5.1*	>100	89.4 (74.4, >100)
EC50 (% test soil in total soil)			
Lettuce Root Elongation	<3.1 *	17.9 (7.6, 23.0)	16.2 (12.6, 20.0)
Oat Root Elongation	12.1 (0, 23)	49.8 * (44.7, 55.7)	44.6 * (35.1, 61.4)

* Response in reference toxicant or negative controls were not in expected range

Case Study 2 - Summary

- Soil Washing was effective
  - Chemistry
    - PCP, Alkanes, and PAHs removed
    - 3- stage process most effective
  - Bioassays
    - Earthworms and plants show reduced toxicity in treated soils.
    - 2-stage process most ecologically hospitable
- Likely that soil washing alters other aspects of soil

Risk Management of EDCs

- Uncertainties of EDCs
  - Unknown endocrine activity of degradation products
  - Unknown effectiveness of treatments in reducing endocrine activity
- Concurrent chemical and biological measures of effectiveness recommended

EDC Bioassays - Considerations

- EDCs of concern in NRMRL projects
- Concentrations
- Environmental Matrices
- Data Quality - Reproducibility and Reliability
- Practicality - Cost and Ease of Use
- Recommendations of Others
- Adaptability to RM projects

EDCs of Concern in NRMRL projects

- Alkylphenols
- Chlorinated Dioxins and Furans
- Estrogens, biogenic and pharmaceutical
- PCBs

All are estrogenic;
some have thyroid and developmental effects

EDC Bioassays - Considerations

- Environmental Matrices in NRMRL projects
  - Air
  - Water
  - Solids - soils, sediments, and biosolids
- Concentrations
  - Water - as low as ng 17 β-estradiol/L
  - solids - levels vary

EDC Bioassays - Considerations

- Data quality
- Practicality
- Adaptability
- Sensitivity

Evaluated by

- Peer reviewed literature
- EDSTAC report
- ORD colleague recommendations

Types of EDC Bioassays Considered

- Sediment/Aquatic Invertebrate tests
- Terrestrial Invertebrate tests
- *In vitro* tests

Fish Vitellogenin mRNA assay through cooperation with MERB/NERL

Sediment/Aquatic Invertebrate tests

- Advantages
  - Commonly studied aquatic organisms
  - Many endpoint options
- Disadvantages
  - Mechanism of action - interference with molting controlled by steroid hormones, ecdysteroids
  - Require substantial lab equipment
  - Test duration - about 1 month

From www.AquacultureStore.com

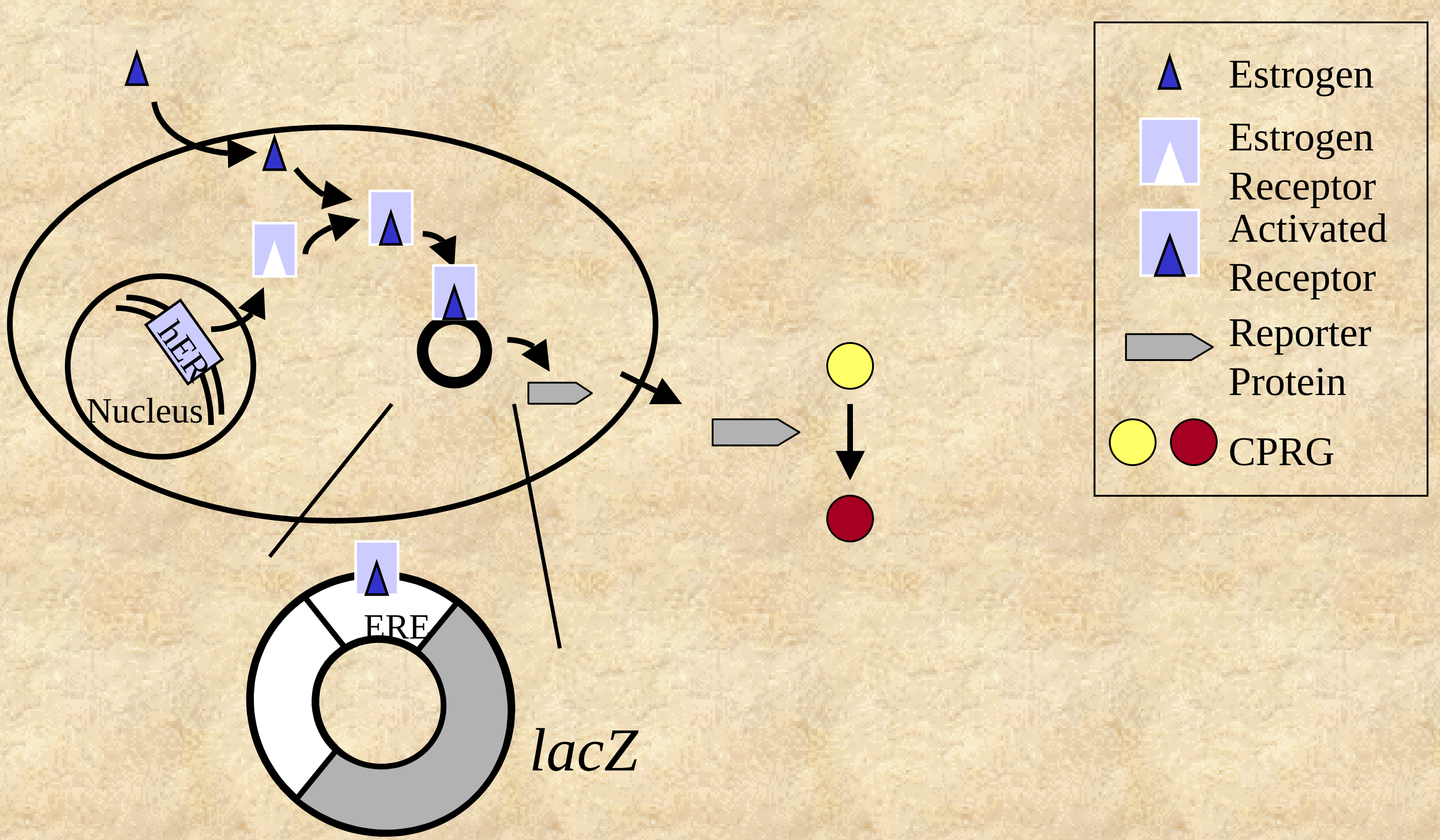
Terrestrial Invertebrate tests

- Imposex Occurrence - measure of androgenicity
- Earthworm Reproduction
  - methods exist
  - endpoints such as number of cocoons and number of hatchlings per cocoon
  - endpoints are not directly related to endocrine function
  - unknown sensitivity to EDCs of concern

In Vitro Assays

- Mammalian cells: E-Screen and MVLN
  - organism - immortal mammalian cell (MCF-7)
  - endpoint - proliferation or luciferase production
  - MVLN - recommended by EDSTAC
- Yeast Estrogen Screening Assay (YES)
  - evaluated by EDSTAC
  - commonly used in peer reviewed literature
  - not recommended for chlorinated pesticides
- Both - reported sensitivity at low concentrations

EDC Bioassay Selected for Adaption - YES Assay



From Routedge and Sumpter, 1996. Environ. Tox and Chem. 15: 241- 248

NRMRL Sponsored EDC RM Projects Using Bioassays

Project	**EDC**	**Prinicpal Investigator**	**Bioassay**
Evaluation of Drinking Water Treatment Techniques for EDC Removal	Steroid Hormones Alkyl Phenols	Kathleen Schenck	MVLN Assay
Potential of CAFOs to Contribute Estrogens to the Environment	Estrogens	Steven Hutchins	FETAX XTRA
Investigations of Sorption and Transport of Hormones and Animal Pharmaceuticals	Estrogens	Suresh Rao Carl Enfield	YES Assay
Evaluating the Fate of EDCs During Wastewater Treatment	Steroid Hormones Alkyl Phenols	Paul McCauley	YES Assay Vitellogenin mRNA Assay
EDCs from Combustion and Vehicular Emissions	PCBs Dioxins/Furans	Brian Gullett	Vitellogenin mRNA Assay
Natural Recovery of PCBs in Sediments	PCBs	Richard Brenner	Vitellogenin mRNA Assay

Using Bioassays in a Hypothetical EDC RM Project

- EDC of Concern - Phthalates
  - commonly used as a plasticizer in many household products (including food containers)
  - suspected to cause alterations in human sexual development
- RM Project - Find a replacement plasticizer for phthalates

Hypothetical EDC RM Project - Phthalate Replacement

- Use computer models to find substances with appropriate chemical and physical properties
- Lab Testing of leading candidates
  - chemical and physical testing to determine if acceptable substitute
  - bioassays to evaluate biological activity
- Look at production processes
  - Are production by-products likely to cause problems?
  - Test bulk chemical - chemical, physical, biological properties

Acknowledgements

- Jennifer Mansfield, IT Corporation
- Kathleen Schenck, U.S. EPA
- Andrew Avel, U.S. EPA

Natural Recovery of PCB-Contaminated Sediments at the Sangamo-Weston/ Twelvemile Creek/Lake Hartwell Superfund Site

Richard C. Brenner[1], Victor S. Magar[2], Glenn Johnson[4], Gregory S. Durell[3], Eric A. Crecelius[5], James E. Abbott[2], Jennifer A. Ickes[2], and Carole S. McCarthy[3]

[1]U.S. EPA, NRMRL, Cincinnati, OH [2]Battelle, Columbus, OH
[3]Battelle, Duxbury, MA [4]University of Utah, Salt Lake City, UT
[5]Battelle, Sequim, WA

Magnitude of Sediment Contamination

- U.S. EPA estimates that 6 to 12% of the sediment underlying the nation's surface water is potentially contaminated (Long *et al*., 1996; U.S. EPA, 1997)
- 720,000,000 to 1,440,000,000 yd^3 of contaminated sediment reside in the upper 5 cm (U.S. EPA, 1997)

Monitored Natural Recovery

- Monitored Natural Recovery (MNR) of sediments is a remedial option that relies on natural environmental processes to permanently reduce risk, and which includes careful assessment, modeling, and monitoring to ensure success (as defined by the RTDF)

- Natural processes most often associated with MNR:
  - Sediment containment through natural capping
    - Requires net depositional areas
  - Contaminant weathering
    - Biological Processes
    - Physical/chemical processes
    - Contaminant sorption/sequestration

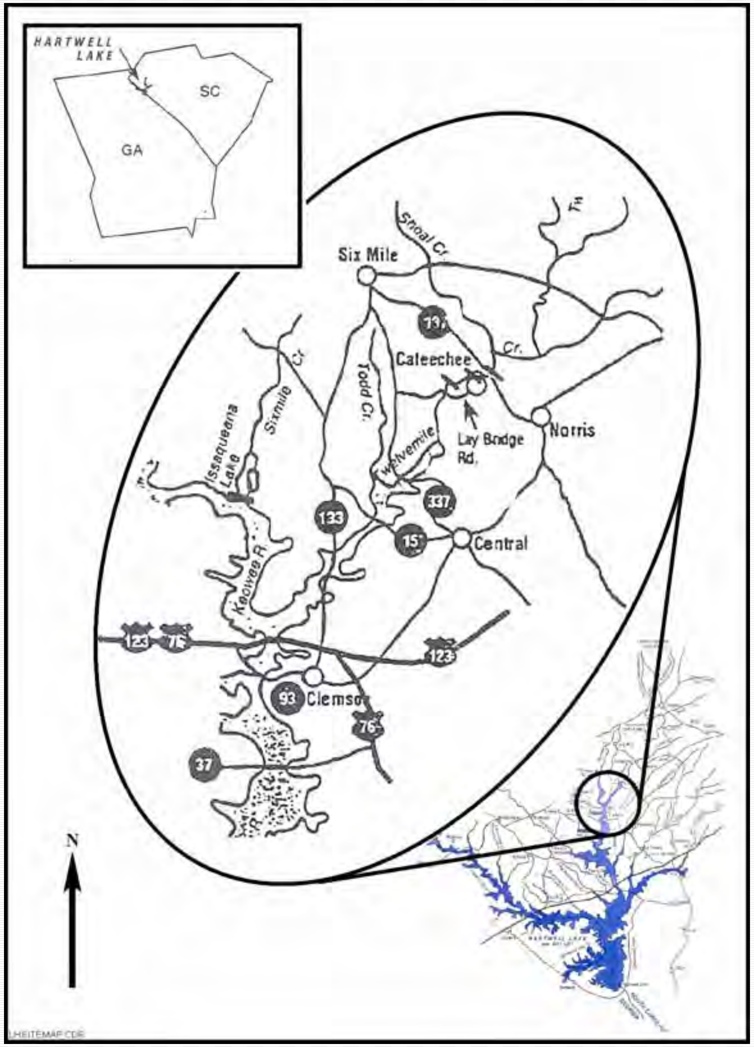
Natural Capping and Burial

- Largest contributor to natural recovery
  - Natural barrier to the aquatic environment
  - Reduces exposure of water column with contaminated surface sediments
  - Reduces upward contaminant diffusion and advection
- Reduces contaminant bioturbation and transport into the food chain
- Potential for resuspension
  - Storm events
  - Construction/industrial activities

Natural Recovery Program Objective

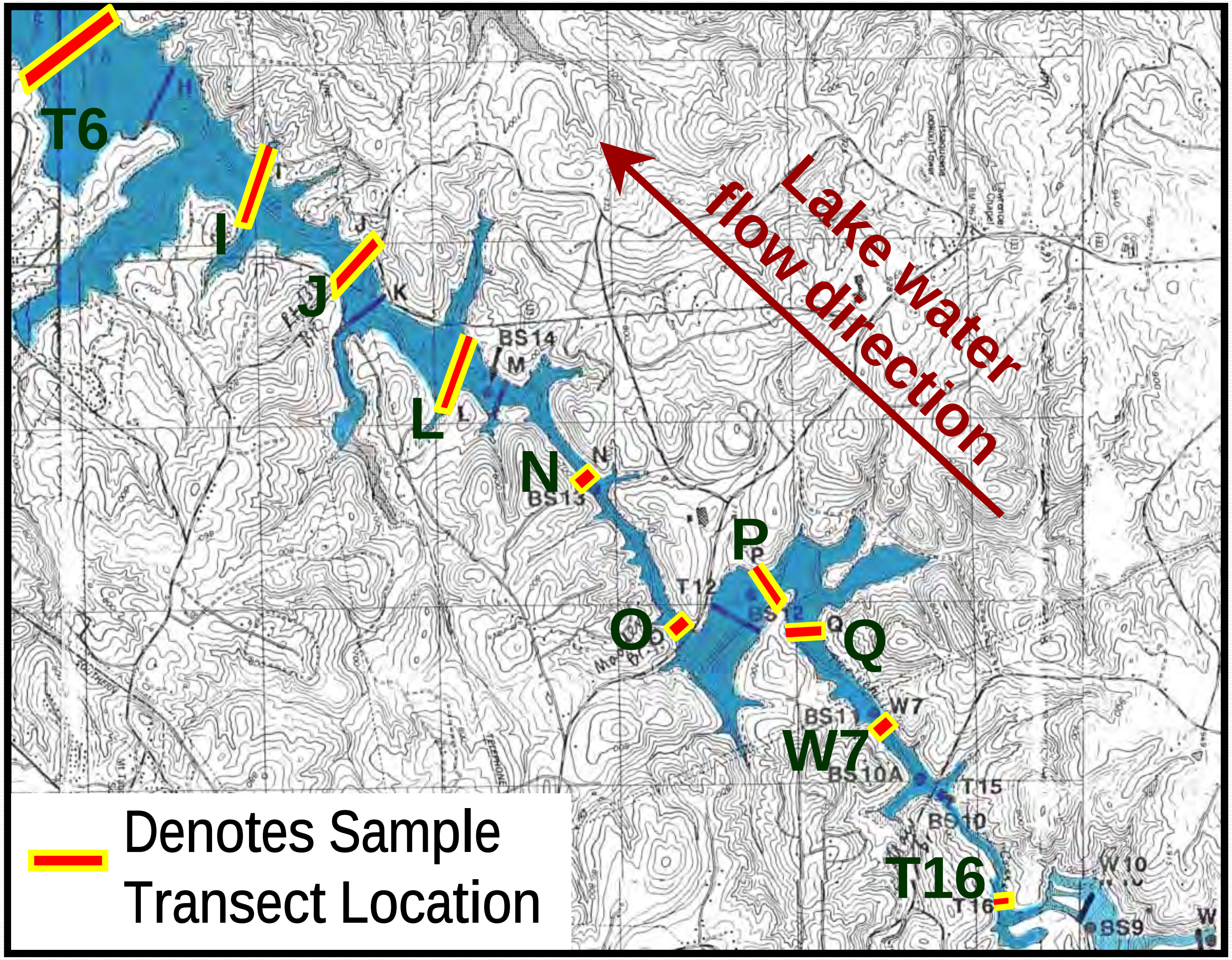
- Investigate natural recovery of contaminated sediments at two sites
  - PCB-contaminated sediments
    Sangamo-Weston/Twelvemile Creek/
    Lake Hartwell Superfund Site (Pickens County, SC)
  - PAH-contaminated sediments
    Wyckoff/Eagle Harbor Superfund Site
    (Bainbridge Island, WA)
- Develop field evaluation techniques
- Use a snapshot approach

Lake Hartwell Site, South Carolina



- Documented history of contaminated sediments
  - Capacitor Manufacturing (1955-1978) – estimated 400,000 tons PCBs discharged
  - Single primary PCB source (Aroclors 1016, 1242, and 1254)
- Natural Recovery selected to restore Lake Hartwell sediments (EPA ROD, R04-94/178)
- Terrestrial PCB contamination has been removed/contained

Lake Hartwell Site Map



- Sediment cores collected at centerline of 10 transects – three cores/transect
- Transects were established by EPA Region 4
- Extruded samples after coring
- **Transect Locations** T16, W7, Q, P, O, N, L, J, I, T6

Upgradient Sample Collection at Lake Hartwell

- Collected sediment cores
- Extruded cores into 5-cm segments

Downgradient Sample Collection at Lake Hartwell

EPA

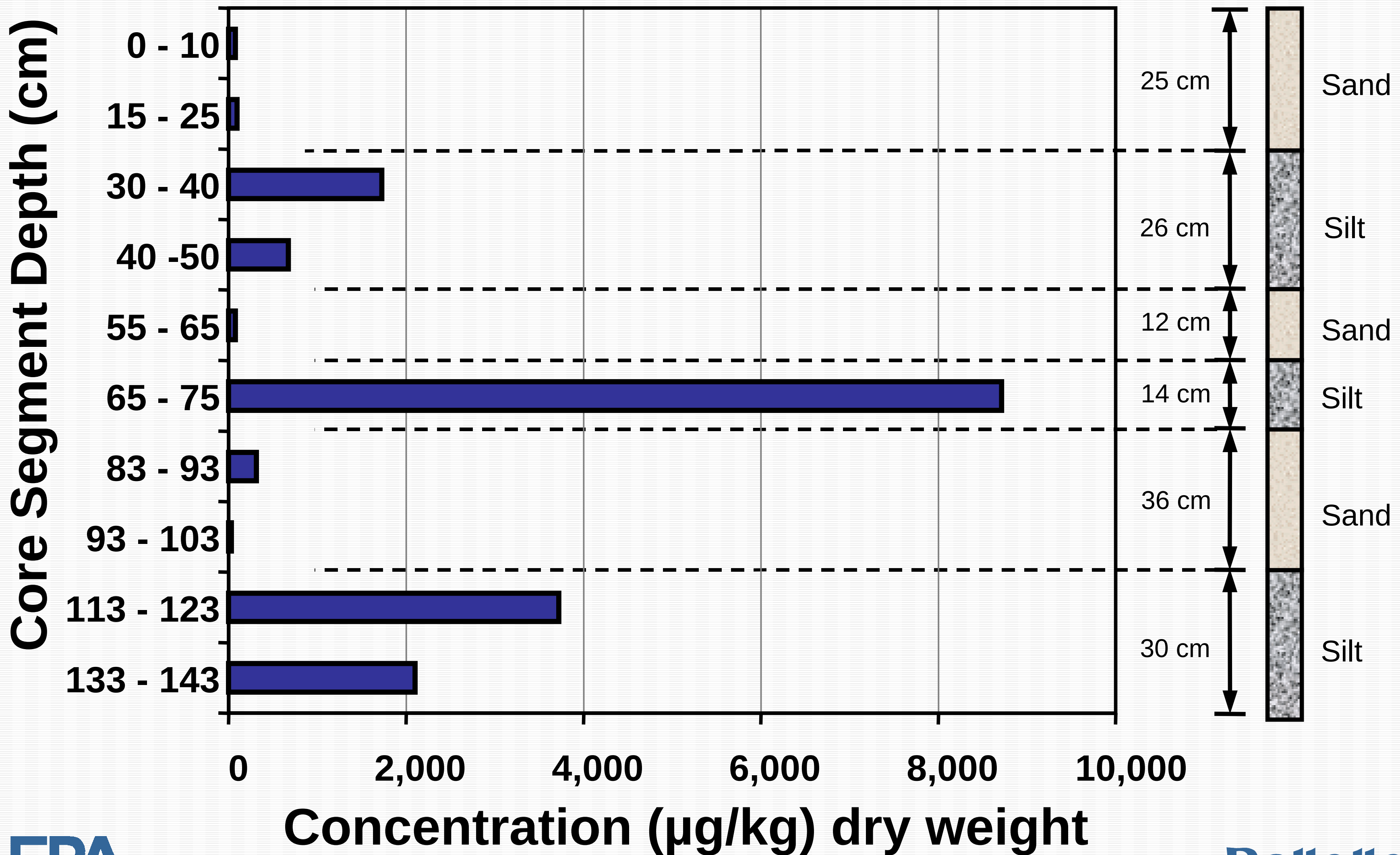
Battelle

Lake Hartwell Results

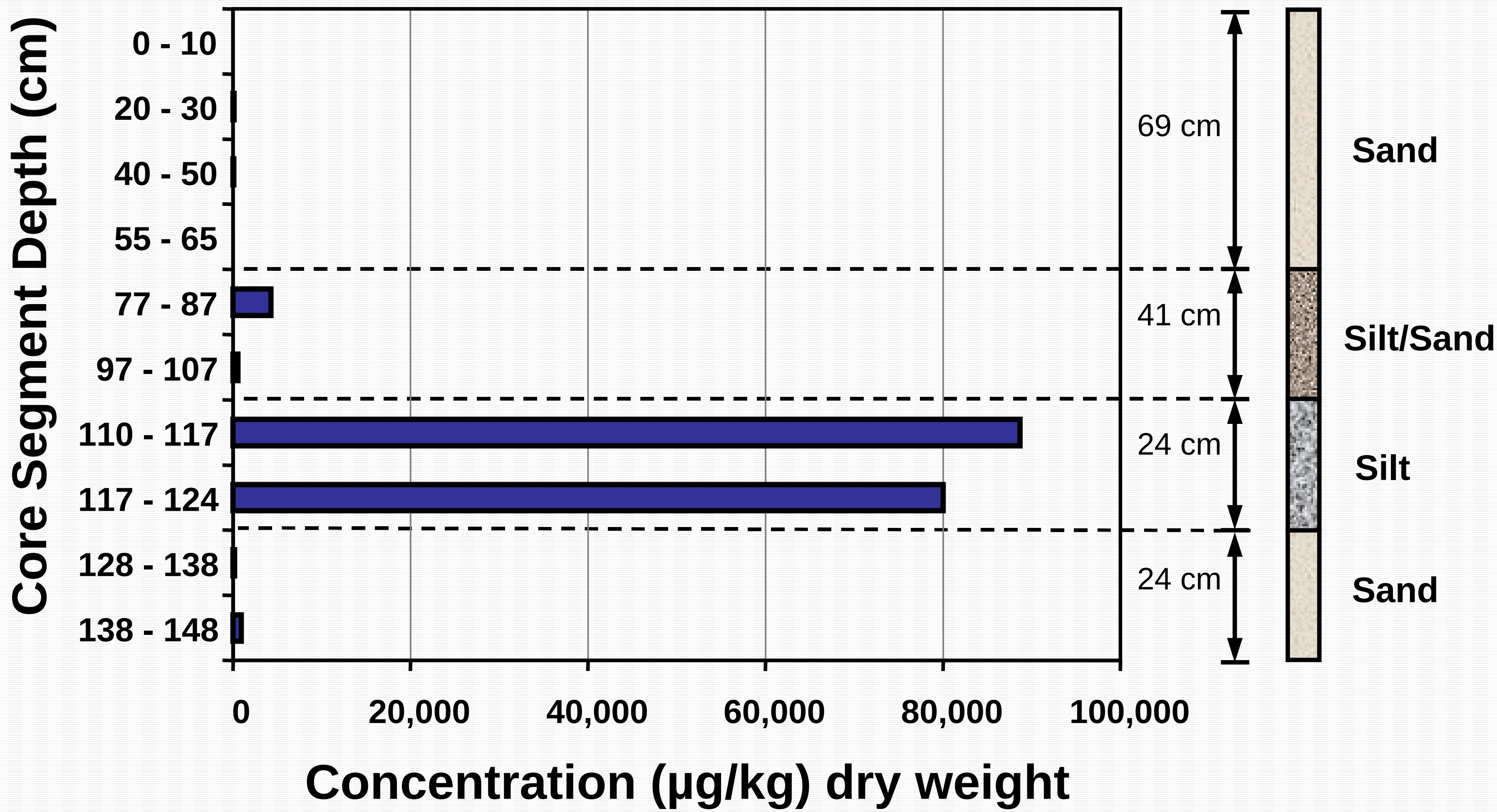
Three sets of results will be discussed:

- Vertical and horizontal PCB distribution
- Sediment age dating [using lead isotope (^{210}Pb) analyses] and sedimentation rates
- PCB homologue and congener distribution analyses

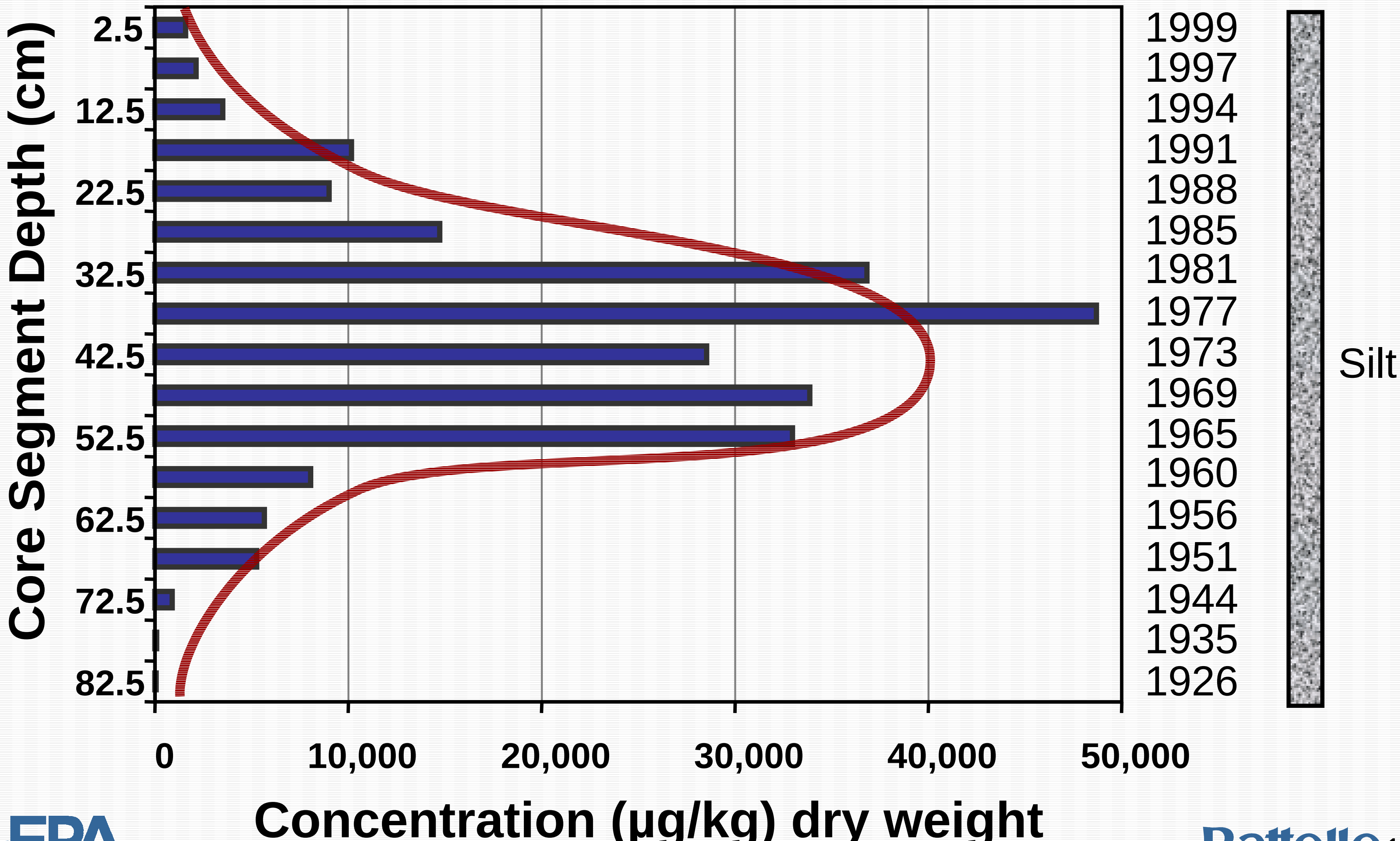
Vertical t-PCB Concentration Profile
Transect Q (Upgradient)



Vertical t-PCB Concentration Profile
Transect T16 (Upgradient)



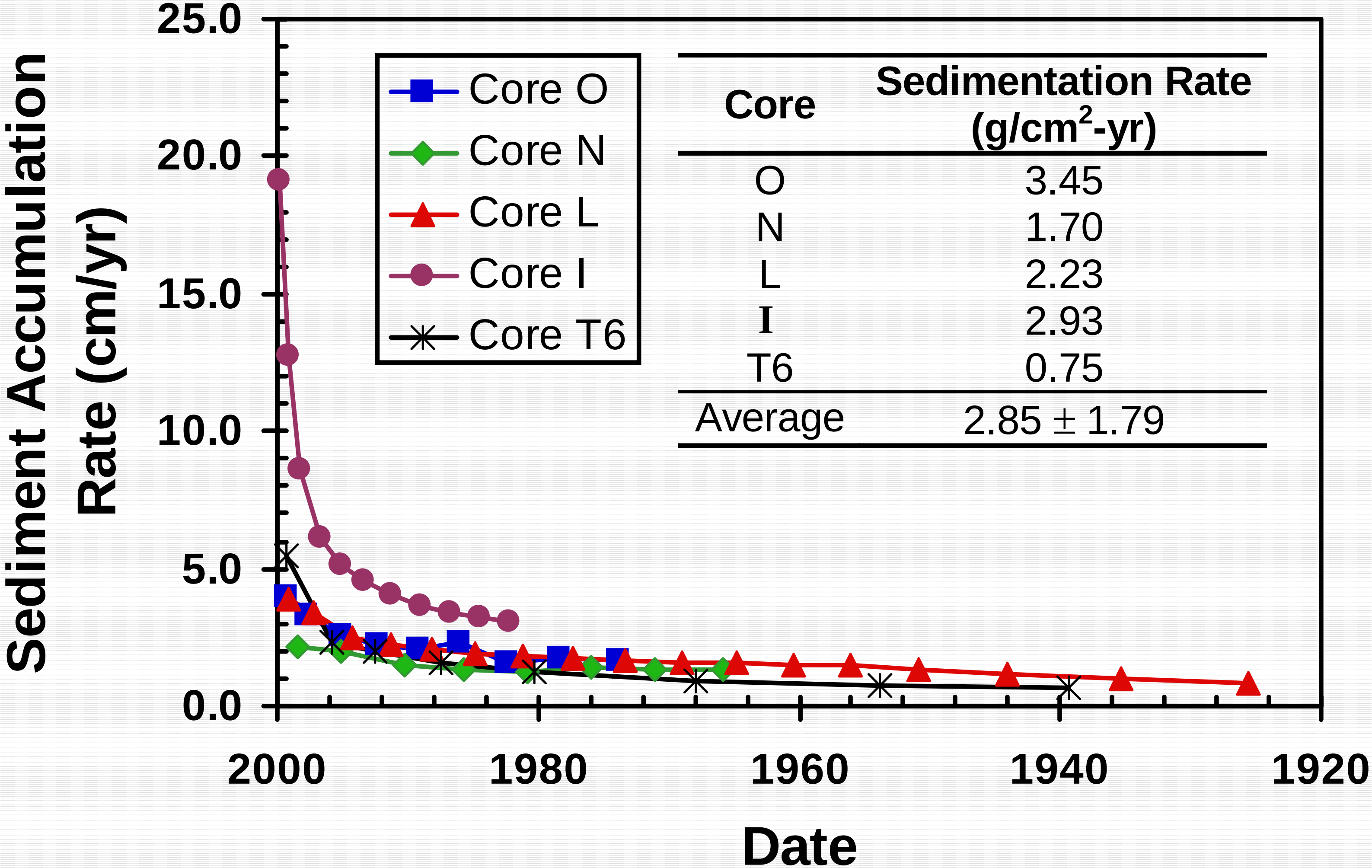
Vertical t-PCB Concentration Profile
Transect L (Downgradient)



^{210}Pb and ^{137}Cs Results

- Assumptions for uniform flux of ^{210}Pb onto surface sediments
  - Uniform grain size throughout sediment profile
  - Constant historical sedimentation rate
- Assumptions met in downgradient Cores O, N, L, I, and T6
- Upgradient cores and Core J could not be dated
- Very low or variable ^{137}Cs concentrations meant ^{137}Cs could not be used to date sediments

Sediment Accumulation Rates



Core	Sedimentation Rate (g/cm^2-yr)
O	3.45
N	1.70
L	2.23
I	2.93
T6	0.75
Average	2.85 ± 1.79

Estimated Additional Sedimentation (cm) Needed to Achieve Sediment Cleanup Goals

Core	1 mg/kg t-PCB	0.4 mg/kg t-PCB	0.05 mg/kg t-PCB
O	2.8	16	45
N	0	7.8	29
L	2.7	11	31
I	0	11	42
T6	0	3.5	13
Avg.	1.2 ± 1.7	10 ± 4.7	32 ± 13

- **1 mg/kg:** ROD surface sediment cleanup goal (EPA, 1994)
- **0.4 mg/kg:** Mean site-specific sediment quality criteria (EPA, 1994)
- **0.05 mg/kg:** NOAA effects range-low (EPA, 1994)

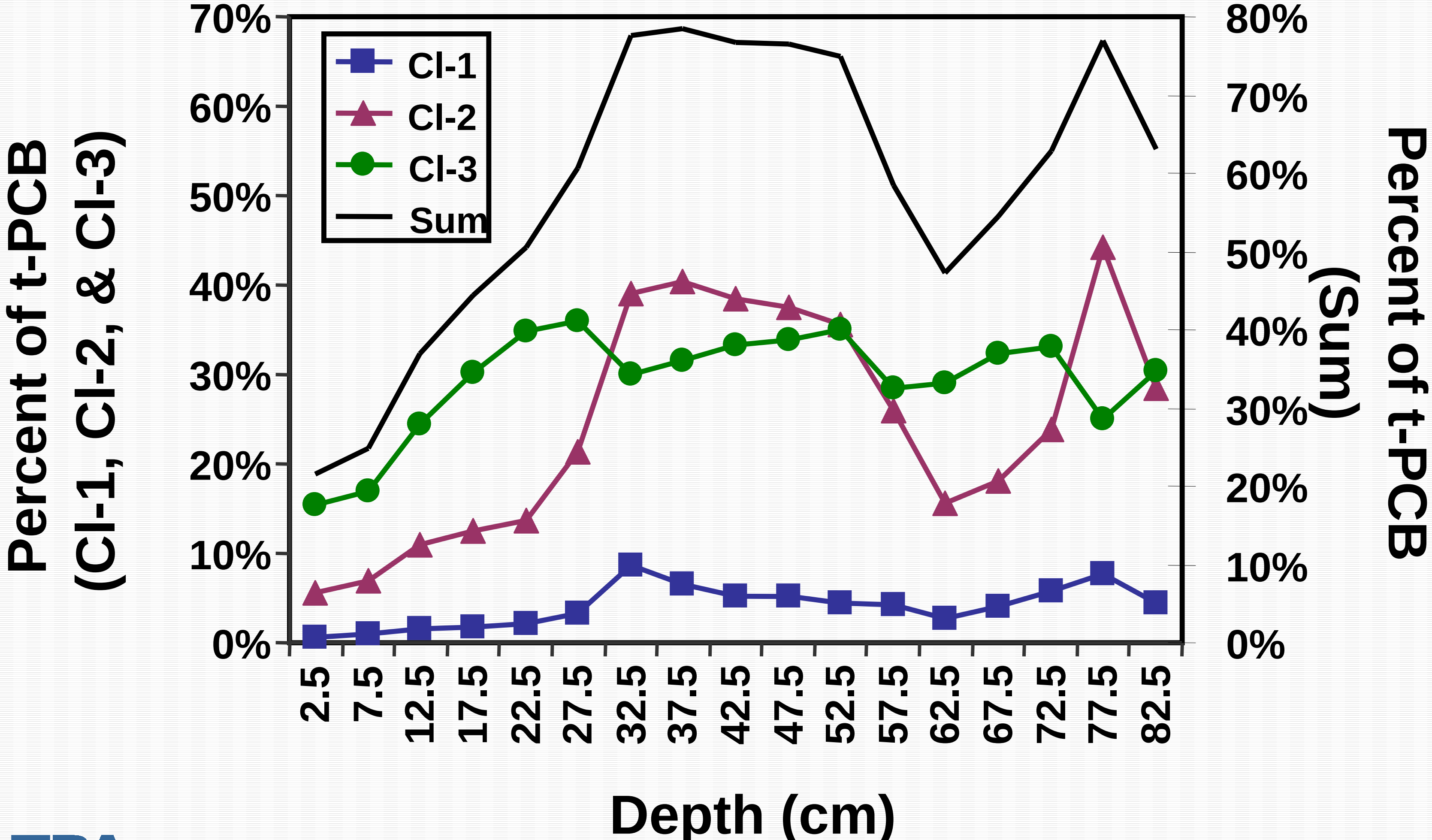
EPA Battelle

Estimated Additional Time (yr) Needed to Achieve Sediment Cleanup Goals

Core	1 mg/kg t-PCB	0.4 mg/kg t-PCB	0.05 mg/kg t-PCB
O	1 - 3	8 - 10	> 28
N	—	5 - 10	25 - 30
L	3 - 5	5 - 7	15 - 20
I	—	2 - 5	10 - 15
T6	—	2 - 5	10 - 15
Range	1 - 5	2 - 10	10 - 30

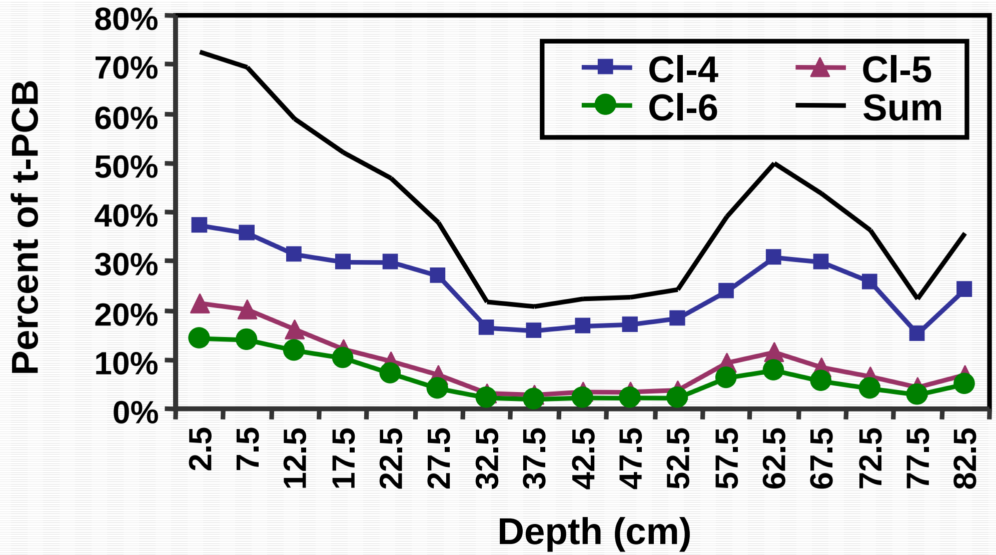
- **1 mg/kg:** ROD surface sediment cleanup goal (EPA, 1994)
- **0.4 mg/kg:** Mean site-specific sediment quality criteria (EPA, 1994)
- **0.05 mg/kg:** NOAA effects range-low (EPA, 1994)

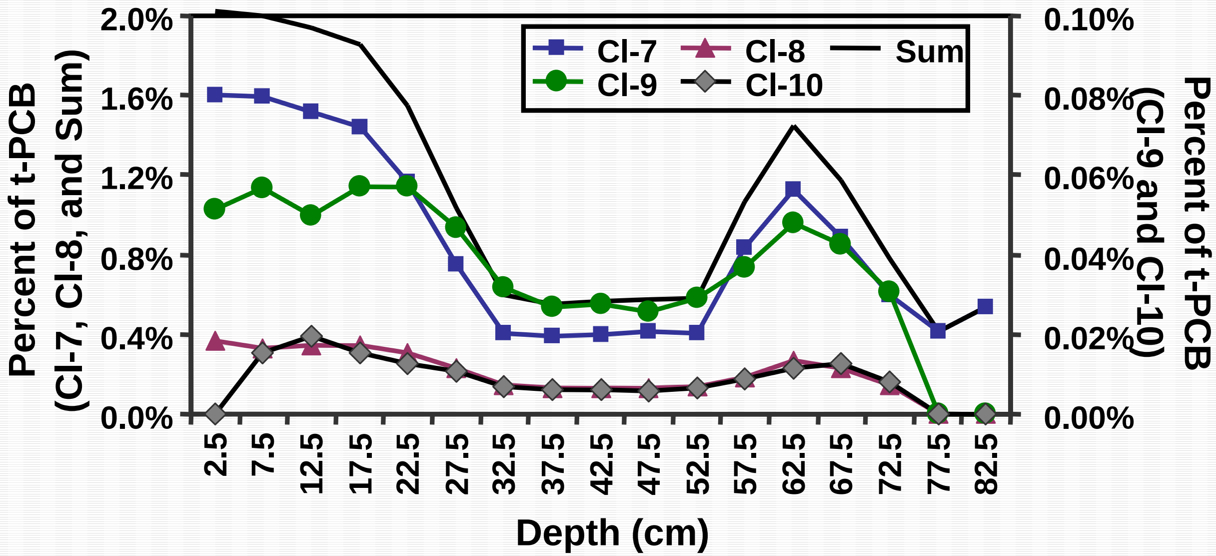
Core L Homologue Plots
Cl-1 through Cl-3



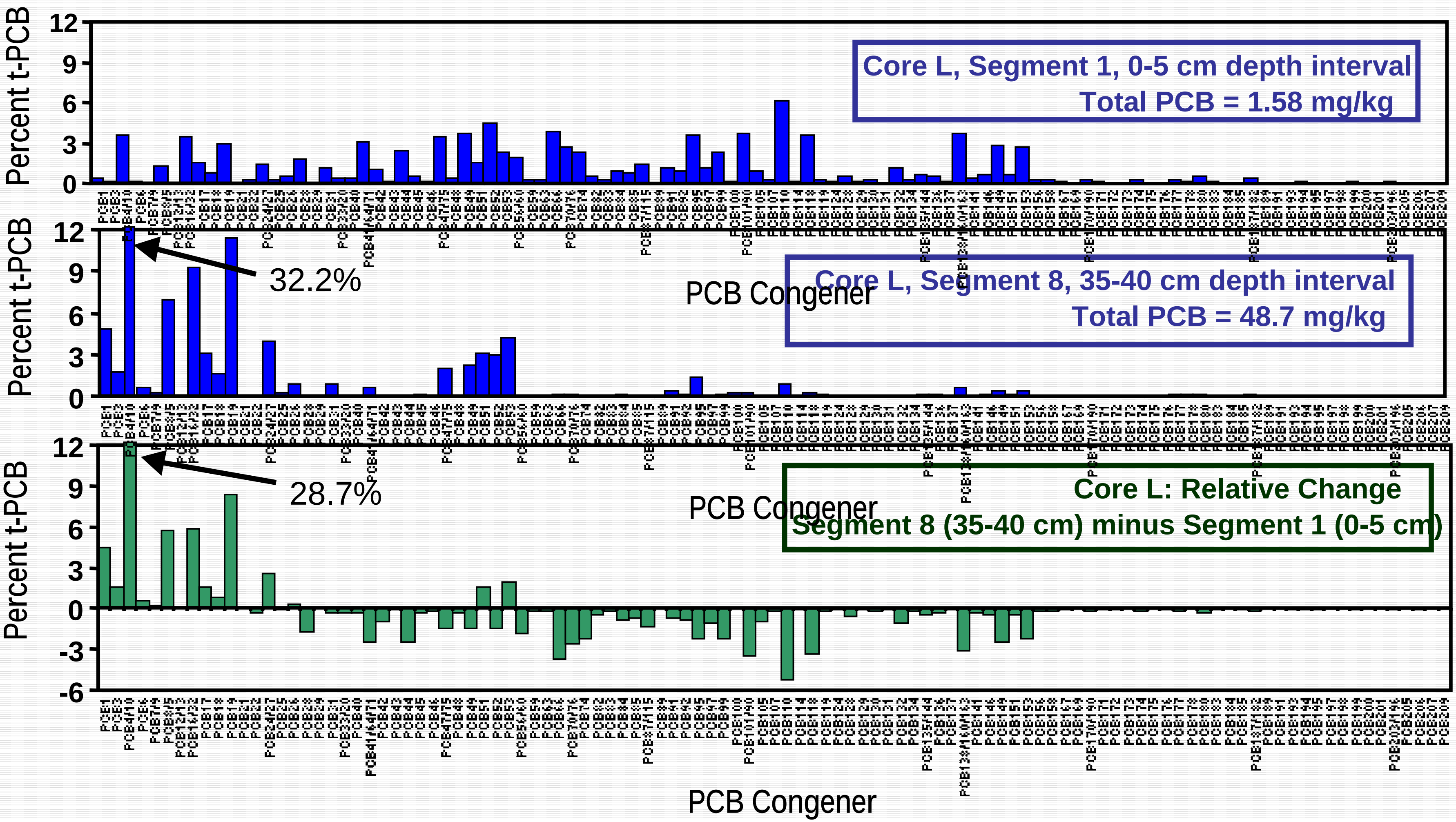
Battelle

Core L Homologue Plots
Cl-4 through Cl-10





PCB Congener Distribution in Surface and Deep Sediments



Summary and Conclusions

- Highest t-PCB concentrations associated with silt/clay layers
- Decreasing surface t-PCBs, at or approaching 1.0 mg/kg (Max. surface PCB concentration = 1.58 mg/kg at Transect L)
- Time to achieve surface sediment concentrations
  - 0 to 5 yr to achieve 1.0 mg/kg
  - 2 to 10 yr to achieve 0.4 mg/kg
  - 10 to 30 yr to achieve 0.05 mg/kg
- Homologue shifts from higher to lower chlorinated congeners
  - Cl4/Cl5/Cl6 congeners reduced from 80% to 20% t-PCB with depth and time
  - Cl1/Cl2/Cl3 congeners increased from 20% to 80% t-PCB with depth and time
- Significant accumulation of ortho chlorinated congeners

Effectiveness of Natural Recovery Approach

Conclusions

- High resolution PCB chromatography (107 congeners eluted) used to characterize vertical PCB concentration profiles and PCB dechlorination patterns with sediment depth and age
- Sediment isotope analyses provided an effective means of calculating sedimentation rates and surface sediment recovery rates

Future Work

- Evaluate relationship of decreasing surface sediment contamination with benthic animals and fish toxicity
- Assess long-term stability of natural recovery progress at this site

EPA Battelle

Removal of Endocrine Disrupting Chemicals from Drinking Water using Granular Activated Carbon

John L. Cicmanec, DVM, MS

Thomas F. Speth, PhD

Kathleen M. Schenck, BS, MS

National Risk Management Research Laboratory, Office of Research and Development

United States Environmental Protection Agency

Office of Research and Development Washington, DC 20460

EPA/625/R-00/015 March 2001 www/epa/gov/ORD

Technology Transfer

EPA

Removal of Endocrine Disruptor Chemicals Using Drinking Water Treatment Processes

Selected Endocrine Disrupting Chemicals

Phthalates

Alkylphenols and AP ethoxylates

Ethinylestradiol

Methoxychlor

Endosulfan

Atrazine

PCBs, Dioxins, and Dibenzofurans

Bisphenol A

Evidence for Endocrine Activity

Compound	Lab	Eco	Human
DDT	X	X	X
PCBs	X	X	X
Dioxins	X	X	X
Alkylphenols	X	X	
Ethinylestradiol	X	X	

Evidence for Endocrine Activity

Compound	Lab	Eco	Human
Methoxychlor	X		
Endosulfan	X		
DEP	X		
DEHP	X		
Bisphenol A	X		

Basis for Selection of EDCs Ecological

DDT	egg shell thinning	Nelson, 1978
PCBs	gulls-sex ratios	Schreiber,1970
Alkylphenols	vitellogenin	Jobling,1995
Dioxins	female-female pairs	Hunt, 1984

Basis for Selection of EDCs Human Data

PCBs reduced birthweights
Patandin,'98, Taylor, '78

Dioxins reduced number of male births
Seveso, Italy

DDT delayed mental development from
***in* utero exposure, Rogan, 1978**

Reproductive Endocrine effects of PCBs (Direct evidence)

Arochlor 1254 in Rhesus monkeys-altered progesterone levels at <u>5</u> µg/kg and <u>80</u> µg/kg (Arnold, 1995)

Arochlor 1016 in Rhesus monkeys-decreased estrogen levels at <u>28</u> µg/kg (Barsotti, 1976)

Reproductive Endocrine effects of PCBs (Indirect evidence)

- **Arochlor 1248-100 µg/kg in Rhesus monkeys 5/8 live births initial gestation as well as residual effects 8, 36, & 55 months later**

"On the Horizon"

Coxsackievirus - juvenile diabetes

Helicobacter pylori - thyroid disorders

Sulfonamide antibiotics - thyroid effects

Trenbolone acetate

Melengestrol acetate

Human Exposure Levels vs Endocrine Effects in Lab Animals

p,p' DDE	
Human fetus, first trimester	11 ppm
Rat fetus, sex differentiation	1-20 ppm
Dioxin	
Human body burden	13 ppt
Rat fetus, sex differentiation	5-40 ppt
MEHP	
Child on dialysis	2.4-15µg/ml
Adult Rat (1 g/kg 14 d)	48-152µg/ml

Environmentally Relevant Doses

Nonylphenol 1000 ug/L. Rhine River

1000 ug/L. Savannah River

75 ug/L. Swedish Rivers

6-160 ug/L. Kansas River

Occurrence of Nonylphenol

- **The concentration of NP in sewage effluent decreased from 75 ug/L. to 9 ug/L. in one year in Sweden**

- **Found in 24% of water samples in U.S.**

Conventional Drinking Water Treatment

- Coagulation
- Flocculation
- Sedimentation
- Filtration
- Chemical disinfection

The Capacity of Conventional Water Treatment Processes to Remove EDCs

- 87% of methoxychlor removed by coagulation with pH adjustment
- 98% of DDT is removed by coagulation
- 35% of PCBs removed by coagulation & filtration
- Removal of lindane, aldrin, dieldrin & parathion varied from 10-60%

Specialized Water Treatment Processes (Organic removal)

Air Stripping

Activated carbon

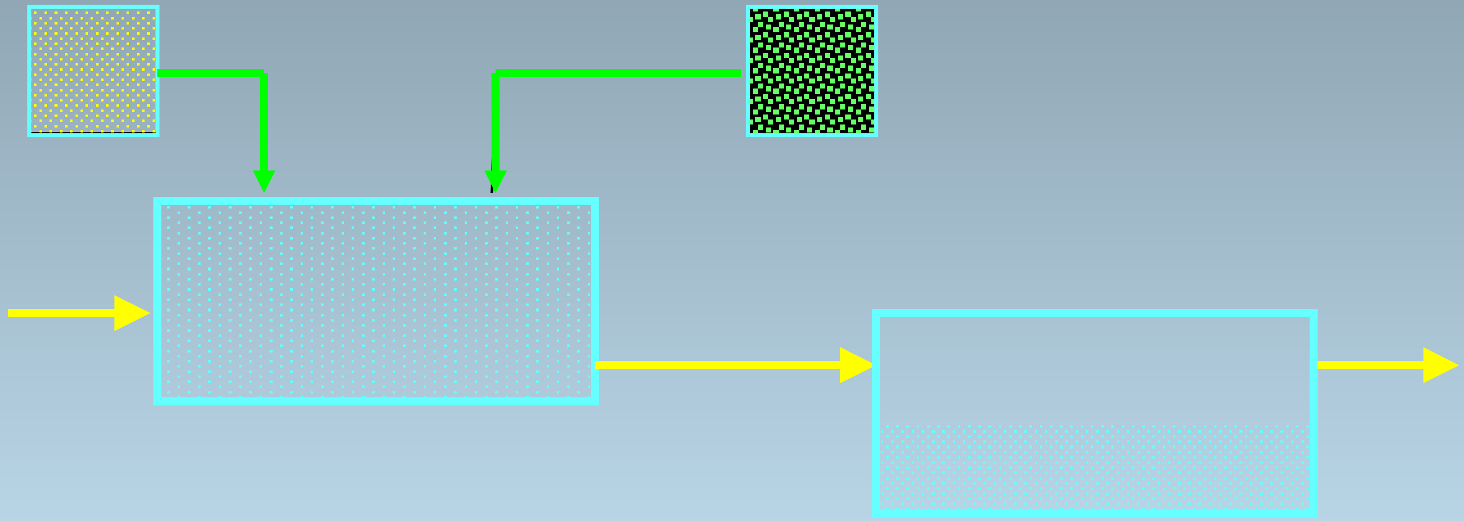
(Granular and Powdered)

Membrane filtration

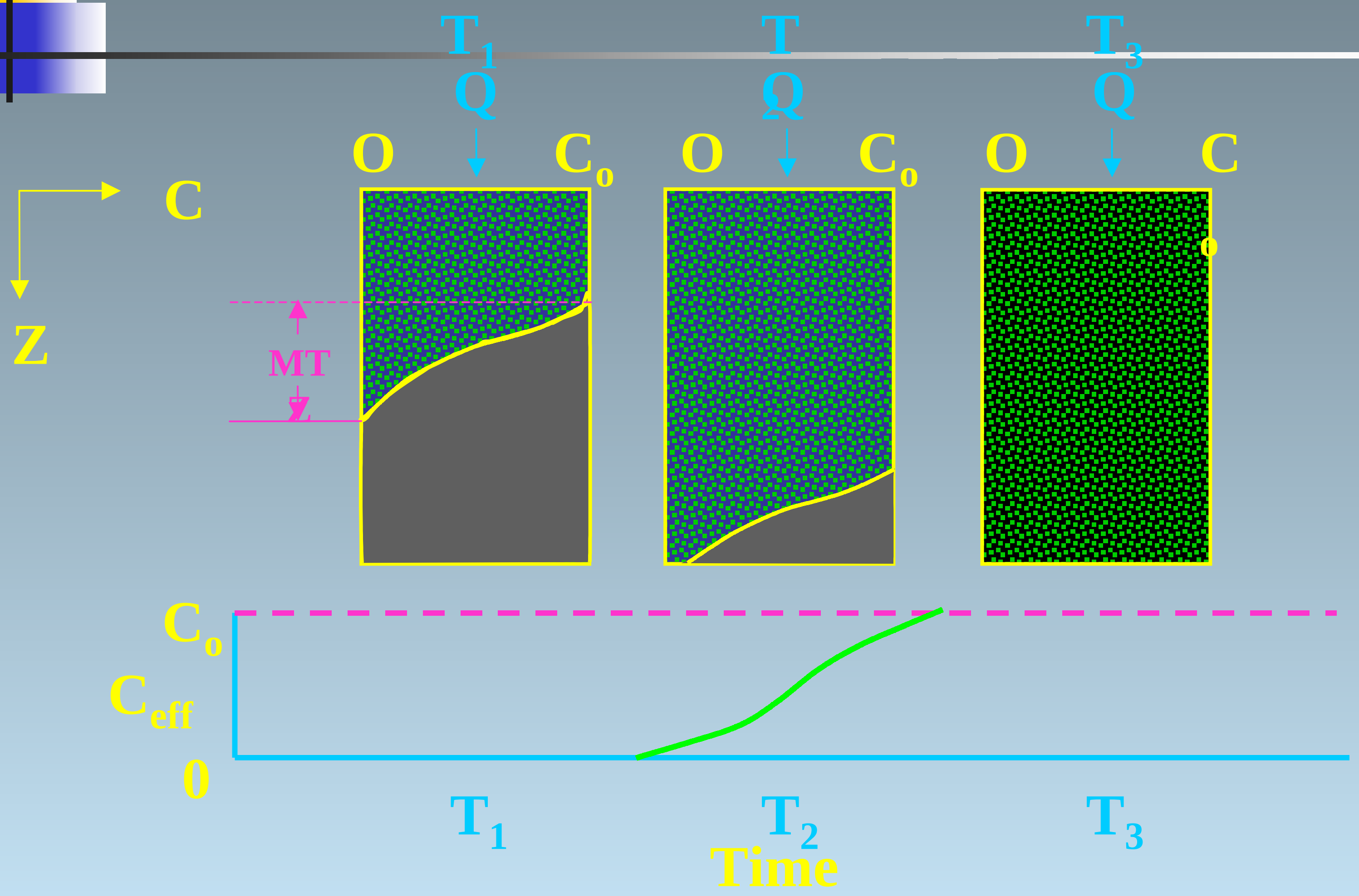
nanofiltration, reverse osmosis

Ultraviolet Light

Powdered Activated Carbon



GAC Adsorption



Present Usage of GAC and PAC (AWWA, 1996)

PAC is used in <u>48%</u> of surface water treatment plants and <u>12%</u> of ground water plants

GAC is used in <u>12%</u> of surface water treatment plants and <u>5%</u> of ground water treatment plants

Factors for PAC/GAC Treatment

Contaminant adsorbability
Concentration
Multicomponent adsorption
Adsorption kinetics
Temperature
Carbon dose
Contact time

Freundlich equation

$$Q = K \times C^{n}$$

Q = Equilibrium capacity of carbon

C^{n} = Equilibrium capacity of liquid-phase concentration of target compound

K and **n** = Freundlich coefficients

Isotherm Constants for Selected EDCs

DDT	10,499
Endosulfan	2,548
Diethylphthalate	17,037
DEHP	8,308
PCB-1221	1,922
PCB-1254	1,050**
PCB-1260	3,700**
Nonylphenol	19,400

Note: Values above 200 are economically feasible

Isotherm Prediction (Speth and Adams, 1993)

$$K = 10^6 * \rho_s * W_o * \exp(\rho_s * B * R * T * \ln(1000 * C_s)/MW)$$

K = Freundlich K in μg/g $(\mu g/L)^{1/n}$
ρ_s = density of pesticide (g/mL) *
W_o = 0.808 mL/g
B = -0.075 mL/cal
R = ideal gas constant (1.987 cal/gmol/^{o}K)
T = temperature (Kelvin)
C_s = pesticide solubility (mg/L)*
MW = molecular weight of pesticide*

Prediction can be off by an order of magnitude!


EXIT


7

Granular Activated Carbon Treatment at CCW

- Present capacity is 200 million gallons/day
- Cost to install granular activated carbon system was $60 million
- Present cost to consumers is 27 cents/1000 gallons vs 25 cents/1000 gallons before

Conclusions

Removal of EDCs by conventional treatment processess is variable

GAC & PAC can remove most hydrophobic compounds such as many of the suspected EDCs

Powdered carbon can remove most EDCs inexpensively and is particularly useful for seasonal application

website

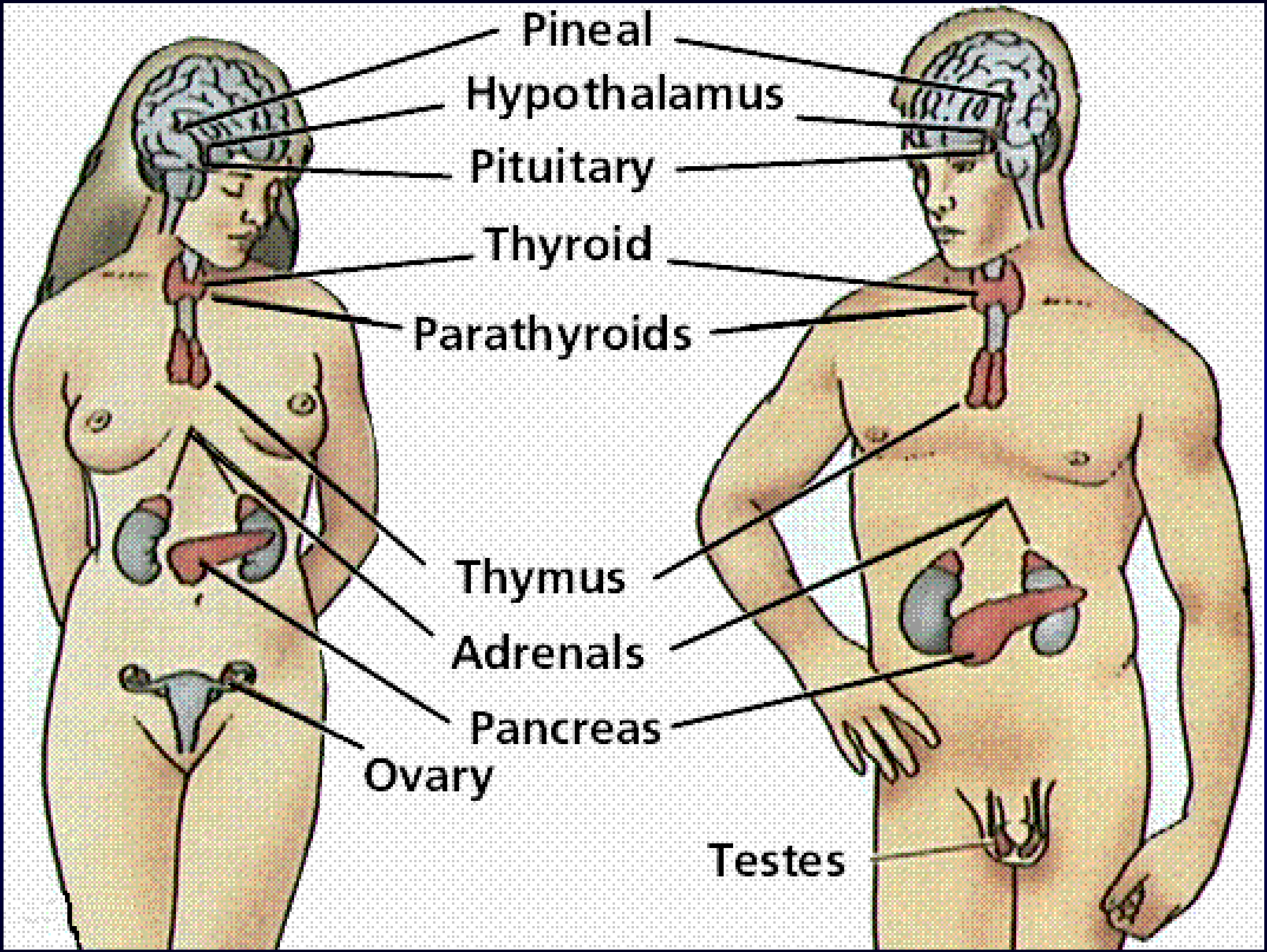
- http:/www.epa.gov/ttbnrmrl

Introduction to EDCs and their Effects on Humans

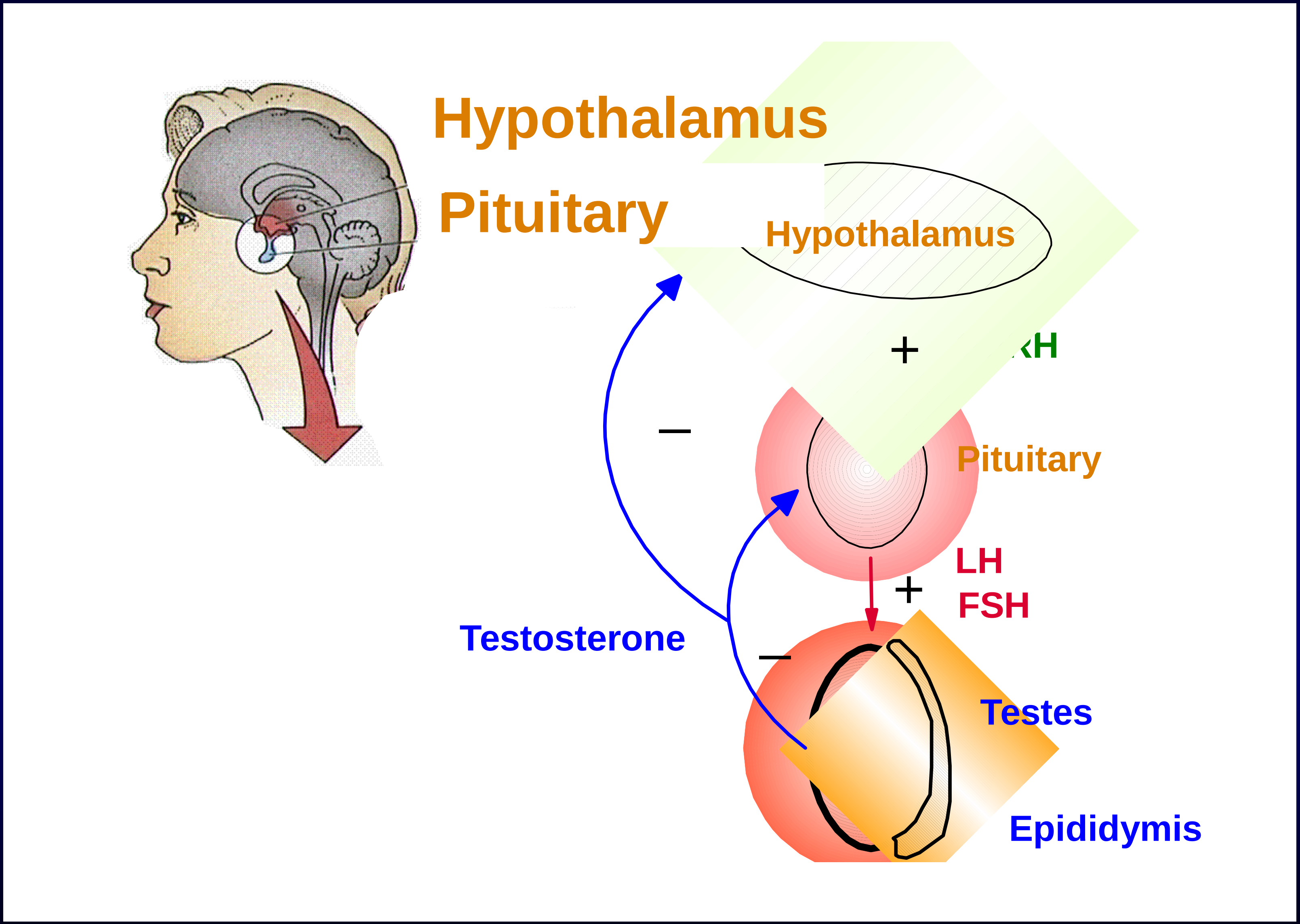
Ralph L. Cooper, Chief
Endocrinology Branch
Reproductive Toxicology Division
National Health and Environmental Effects Research Laboratory

Overview

- Introduction to Endocrine System
- Research Findings
  - Lines of Evidence, Outcomes, Modes, Chemicals
- Endocrine Disruptor Screening Program
  - Mammalian Tests

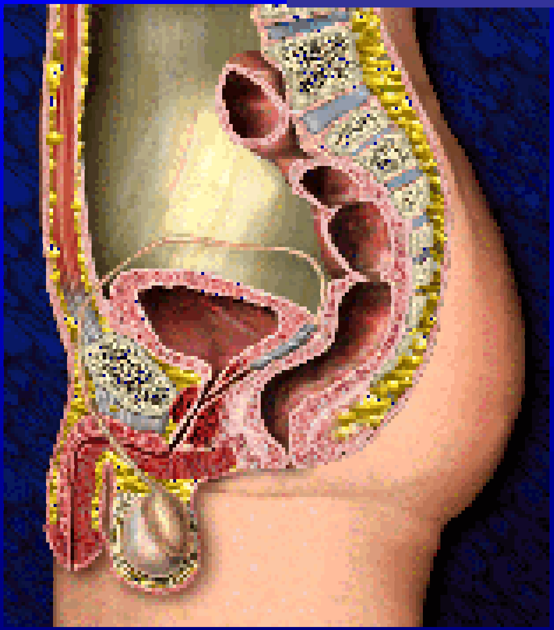

Pineal
Hypothalamus
Pituitary
Thyroid
Parathyroids
Thymus
Adrenals
Pancreas
Ovary
Testes

Hormones of Male Reproduction

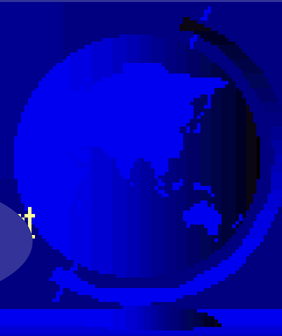


Male Reproductive System

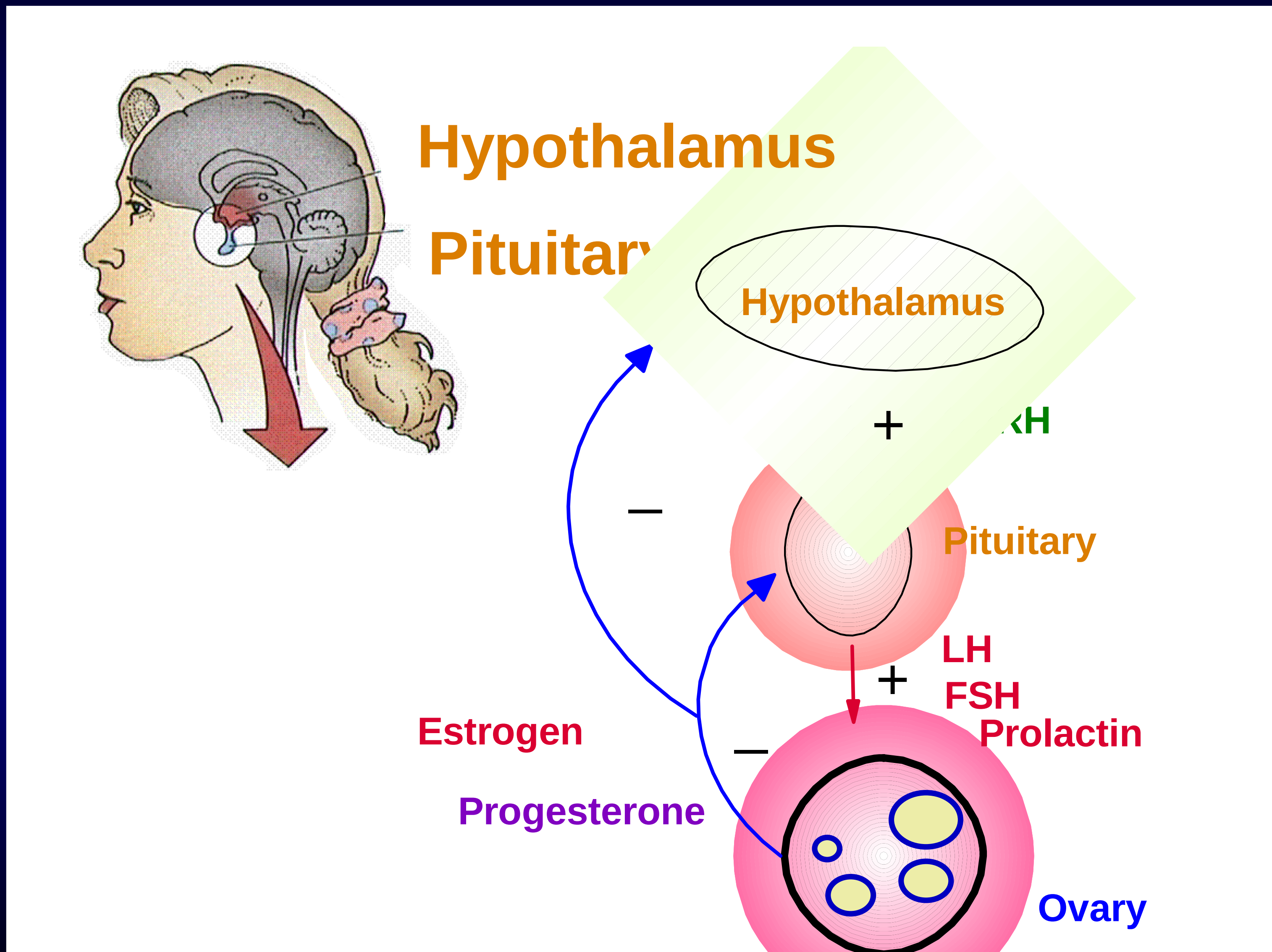
Accessory Organs



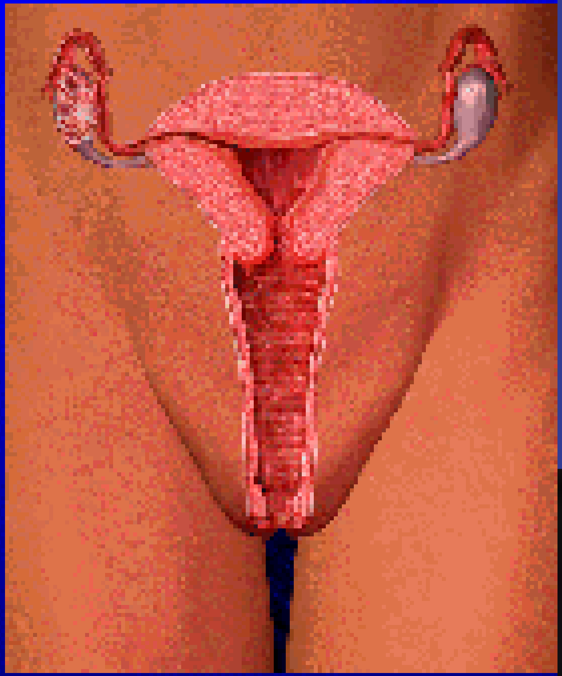
- Seminal Vesicle
- Ejaculatory duct
- Prostate gland
- Bulbourethral gland
- Vas deferens
- Epididymis
- Scrotum
- penis



Hormones of Female Reproduction

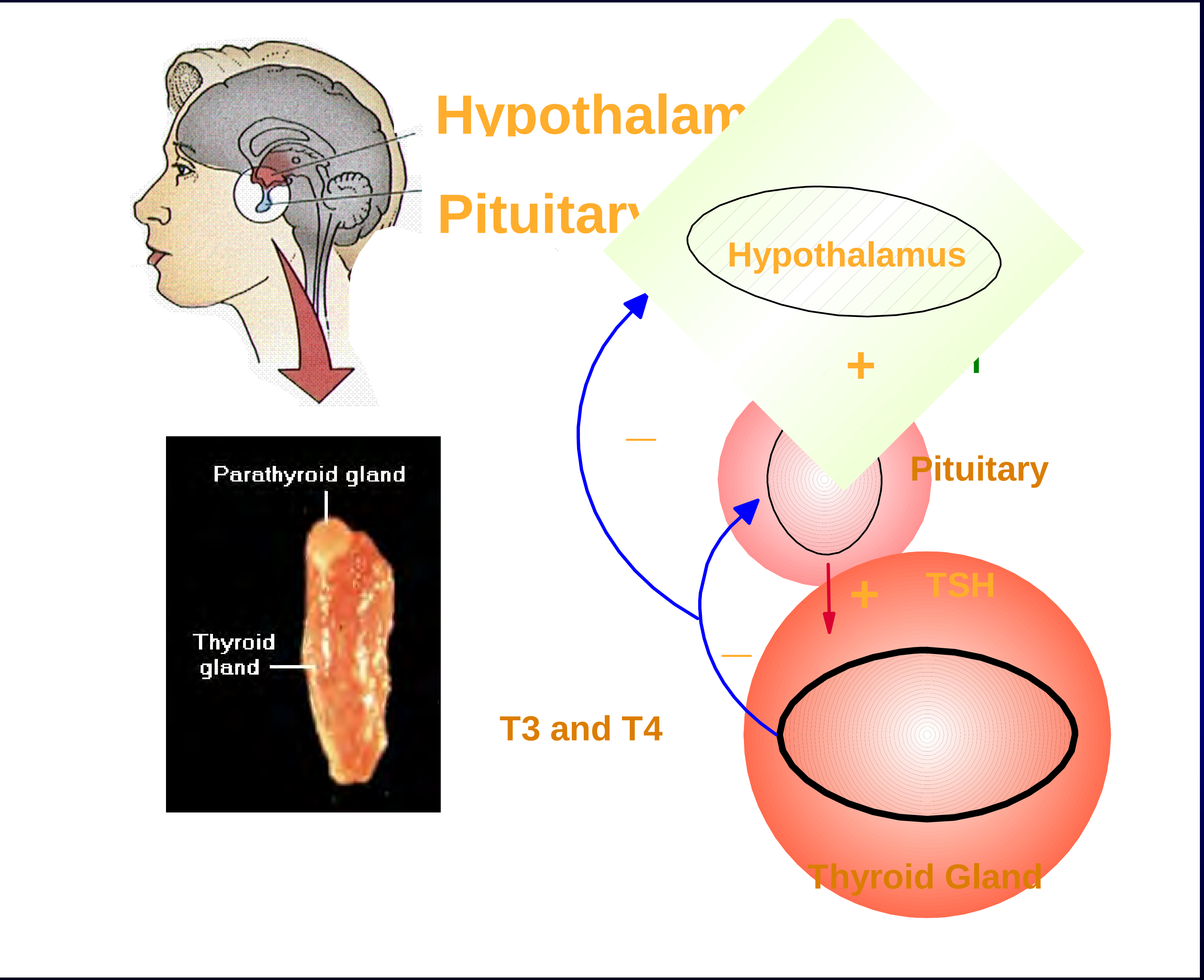


Female Reproductive System



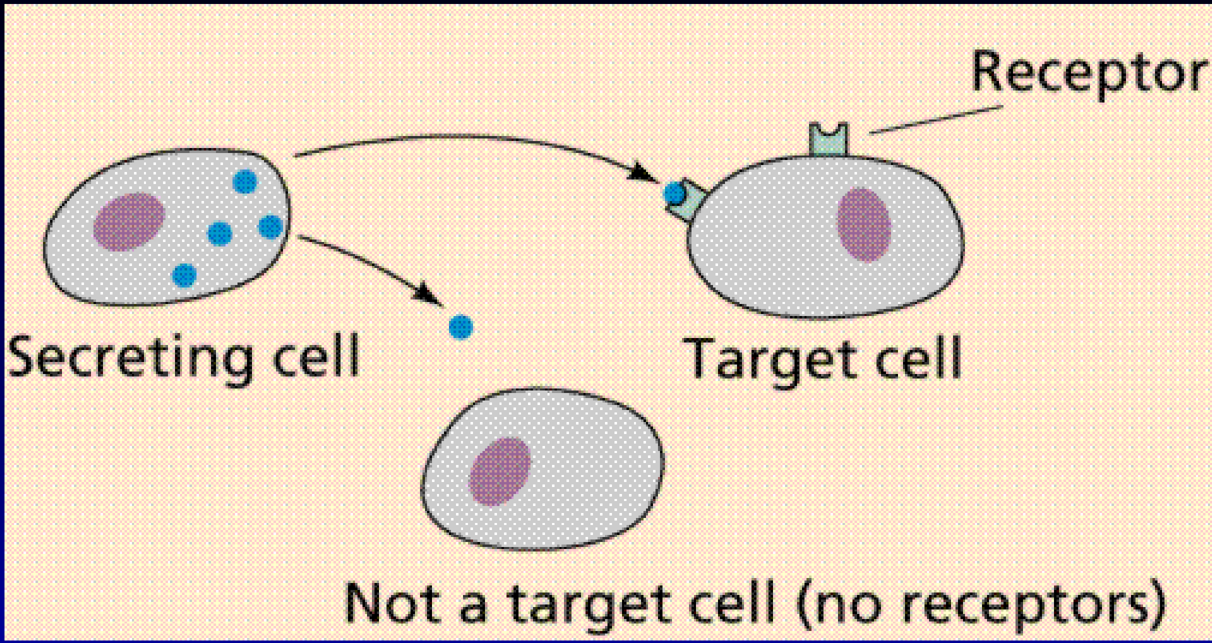
- Ovary
- Uterine Tube
- Uterus
- Cervix
- Vagina

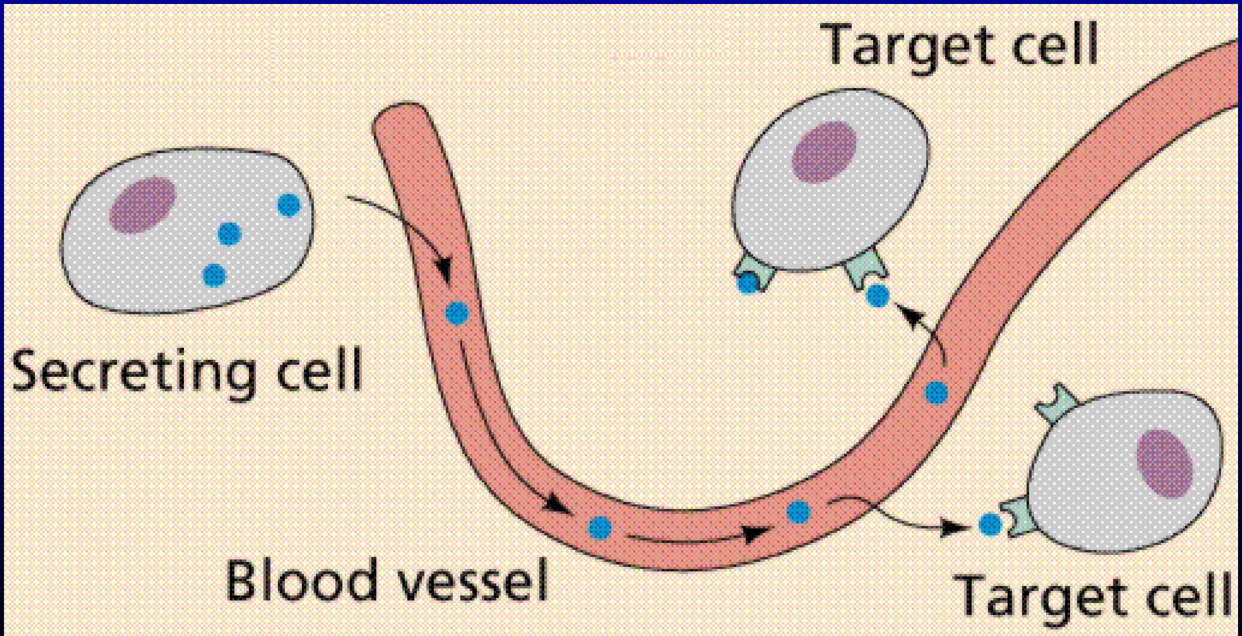
Thyroid Hormone Regulation



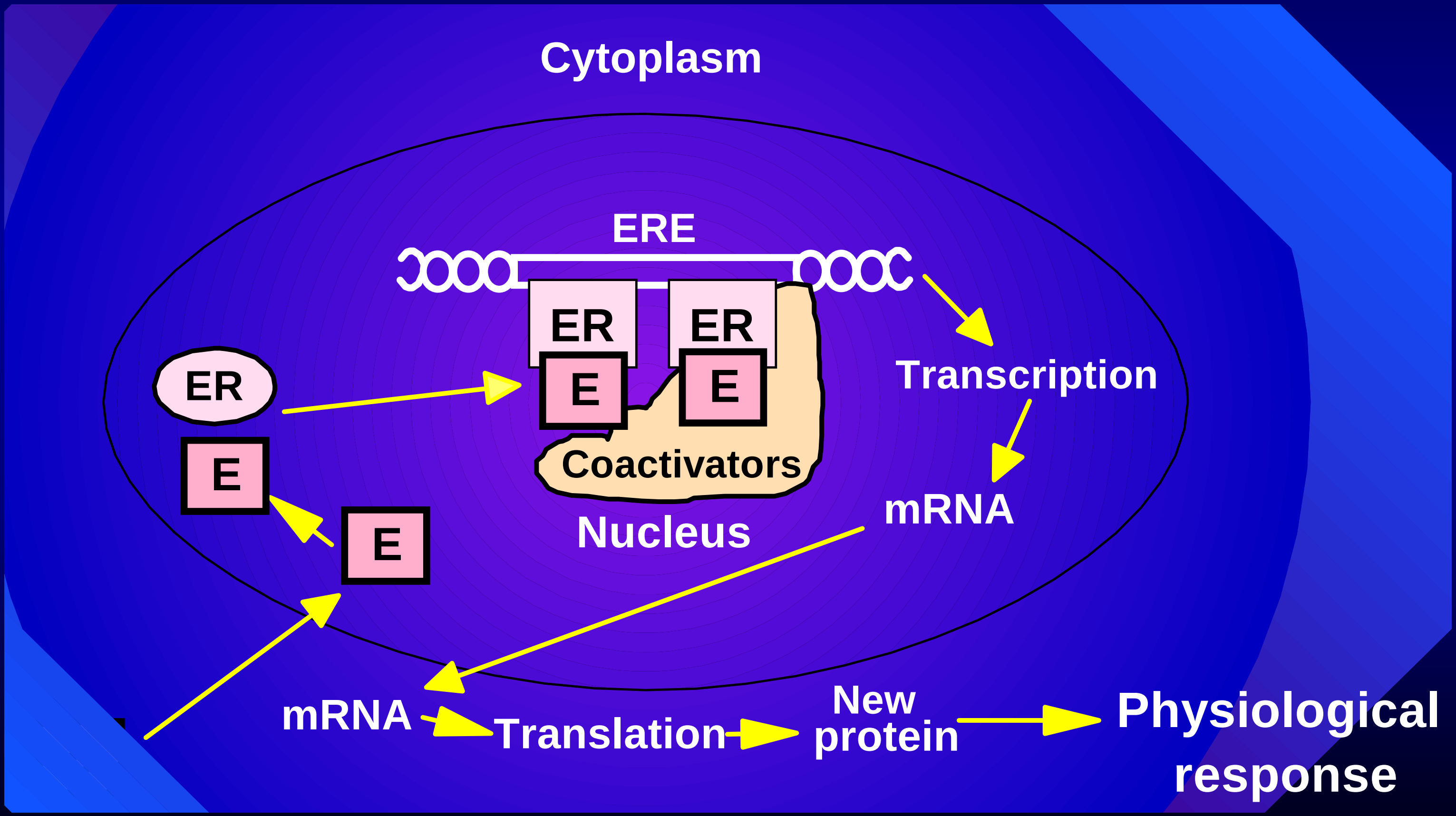
Communication

Cell-to-Cell and
Organ to Organ



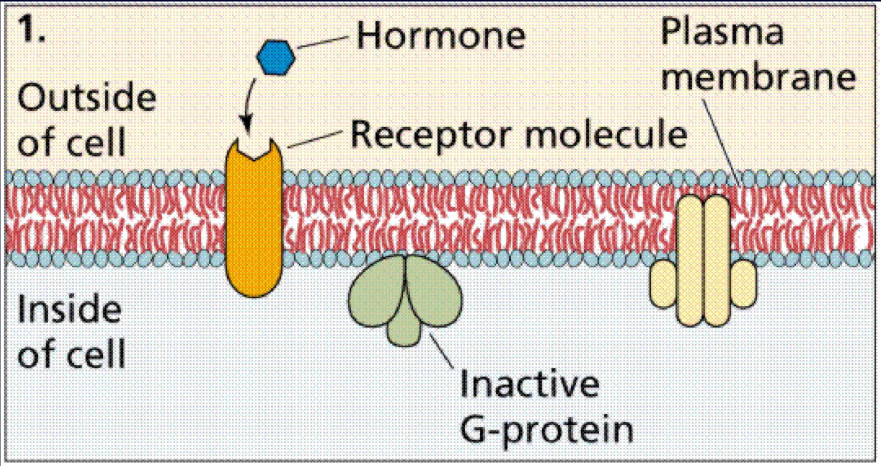


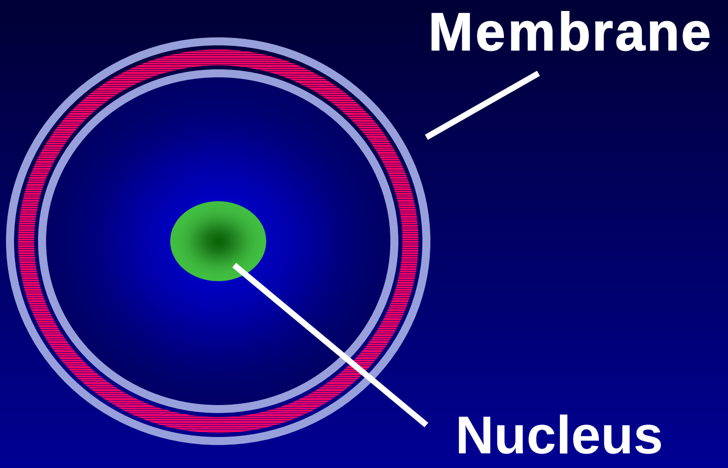
Classical Pathway for Estrogenic Activity Direct Gene Induction

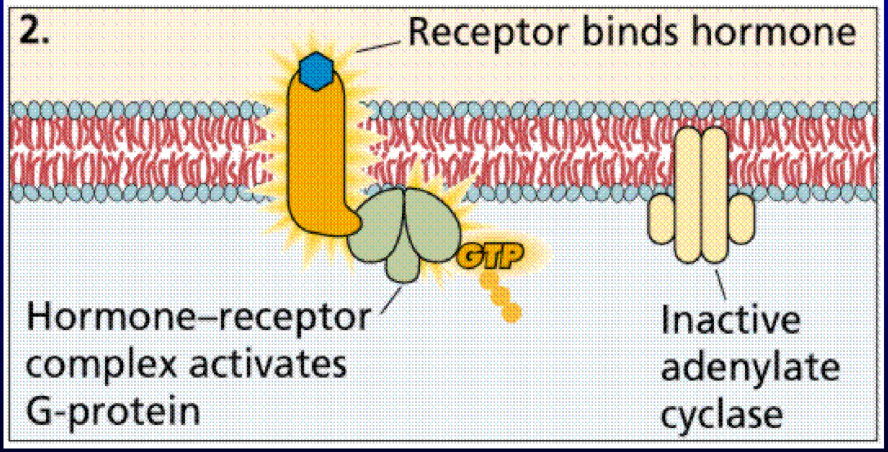


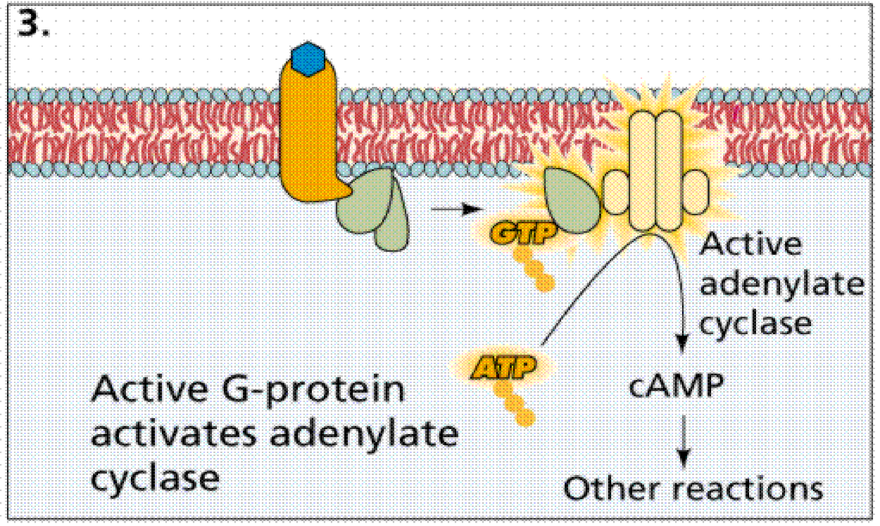
Membrane Receptors

(protein hormones)

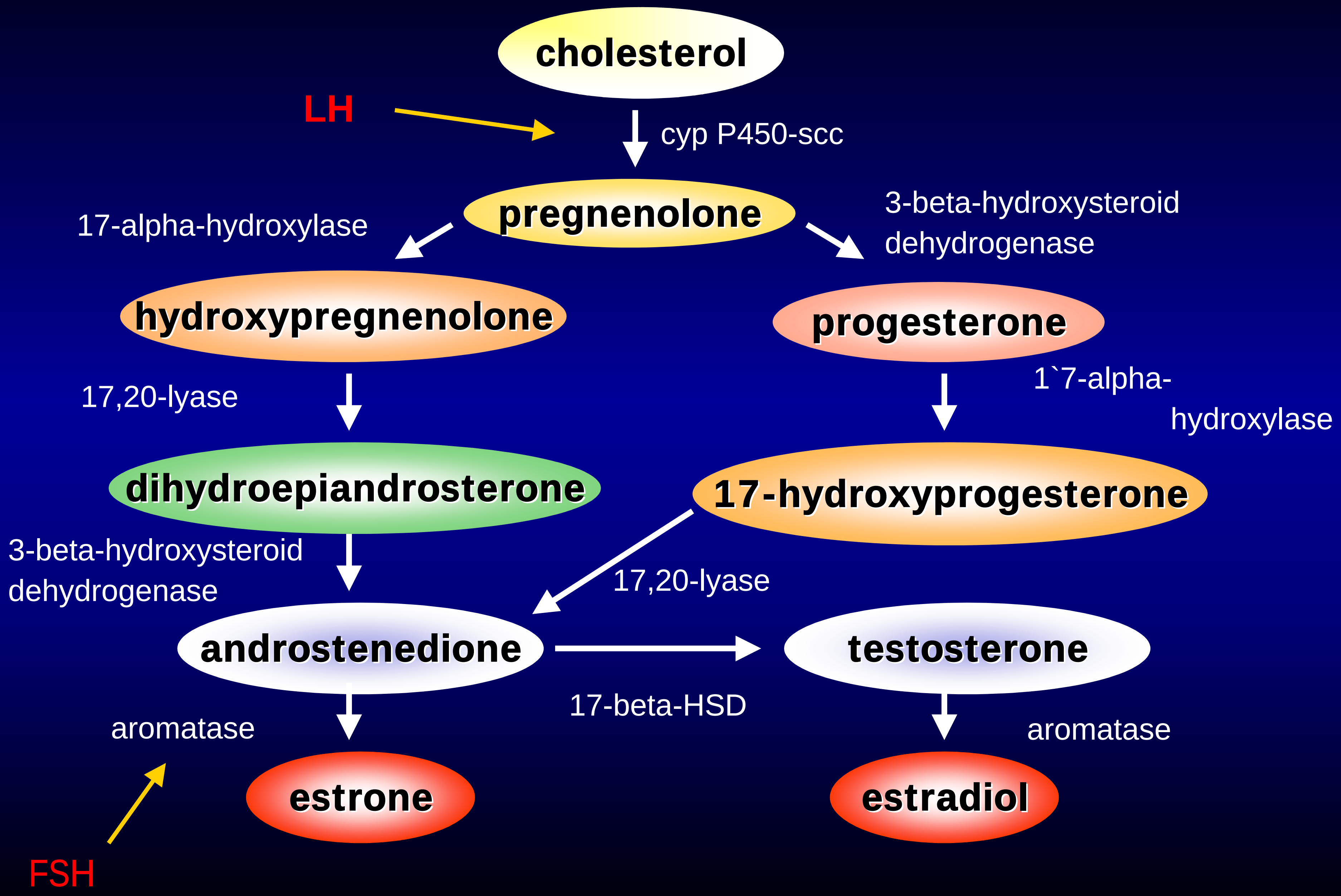








Steroidogenesis



Summary

Endocrine system is a complex network of coordinated regulatory changes.

Homeostatsis

Endocrine system provides a variety of targets for enviornmental chemicals

- Receptors (nuclear and membrane)
- Synthesis
- Clearance

Endocrine Disruptors and Human Health

Definition

- **An exogenous substance that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.**

IPCS, 1998 (Global Assessment)

Modes of Action

Homologous Mechanisms

- Receptor binding
  - ER, AR, AhR, GR, GABA
    - Agonists and Antagonists
- Enzyme Inhibition
  - Steroidogenesis, thyroid peroxidase
- Enzyme induction
  - CYP450s, thyroxine conjugation
- Signal transduction pathways
  - Phosphatases/kinases, transcription factors

Lines of Evidence

- Laboratory based toxicology studies
  - Anti-androgens, estrogens, chlorotriazines
- Field Studies
  - Lake Ontario Lake Trout and dioxin (TEQs)
- Status and Trend Studies
  - Population status, tumor incidence, contaminant loads
- Epidemiology
  - PCBs and developmental neurotoxicity
- Developmental Susceptibility
- Each with Strengths and Limitations
  - Cost, Sensitivity, Predictiveness

Experimental Evidence

- Effects on Developing Reproductive Tract
  - Estrogens (DES, Methoxychlor, Bisphenol A)
  - Anti-Androgens (Vinclozolin, DDE, Phthalates)
  - Ah Receptor Agonists (dioxin, PCBs)
  - Steroid Synthesis Inhibitors
- Developmental Neurotoxicity
  - OH- PCBs (hypothyroidism),
  - Estrogens (masculinization)
  - Polybrominated diphenylethers (hypothyroidism)
- Tumors
  - Thyroid Gland
  - Leydig Cell Hyperplasia
  - Mammary Tumors (Atrazine)

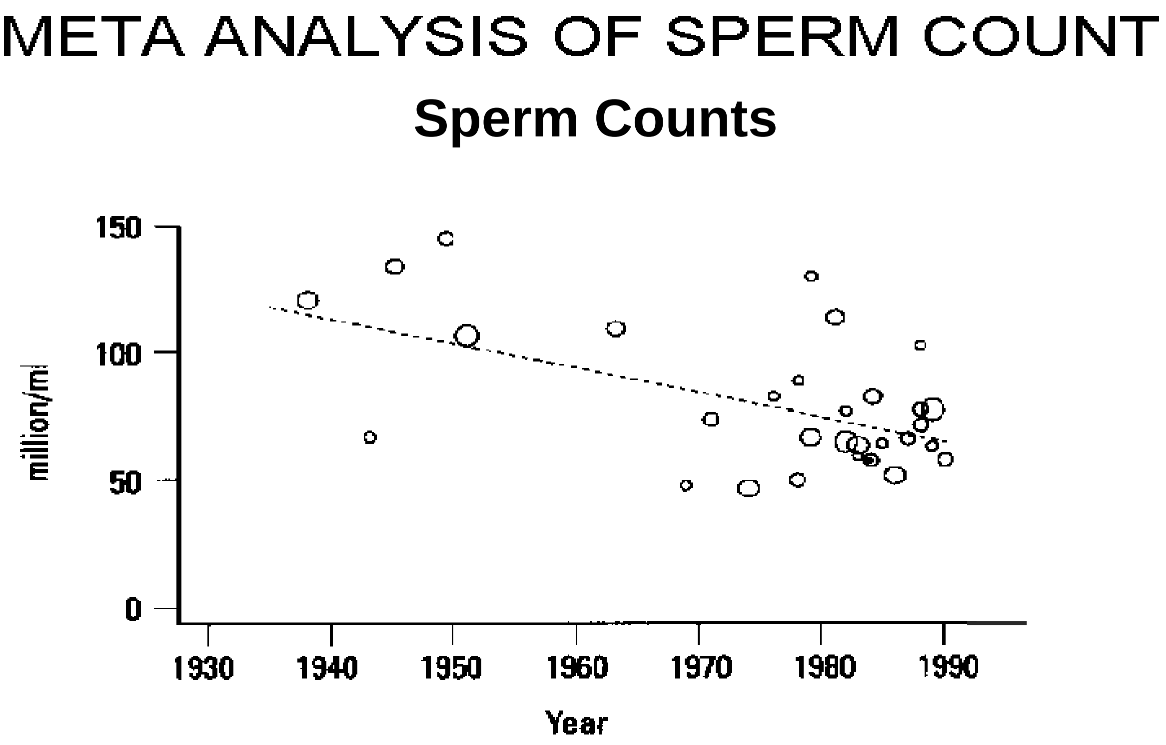
Phenotypes in Exposed Offspring

- Estrogens
  - Females >> Males
  - Brain masculinization
  - Altered Puberty (>f, <m)
  - Reduced Fertility
  - Reproductive Tract Anomalies, Cancers (DES)
- Anti-Androgens
  - Males >>> Females
  - Feminized genitalia, Hypospadias, Nipple Retention, Organ growth
- Ah Agonists
  - Male > Females
  - Reduced sperm release, Vaginal threads, Accelerated eye opening

Reported Human Health Effects

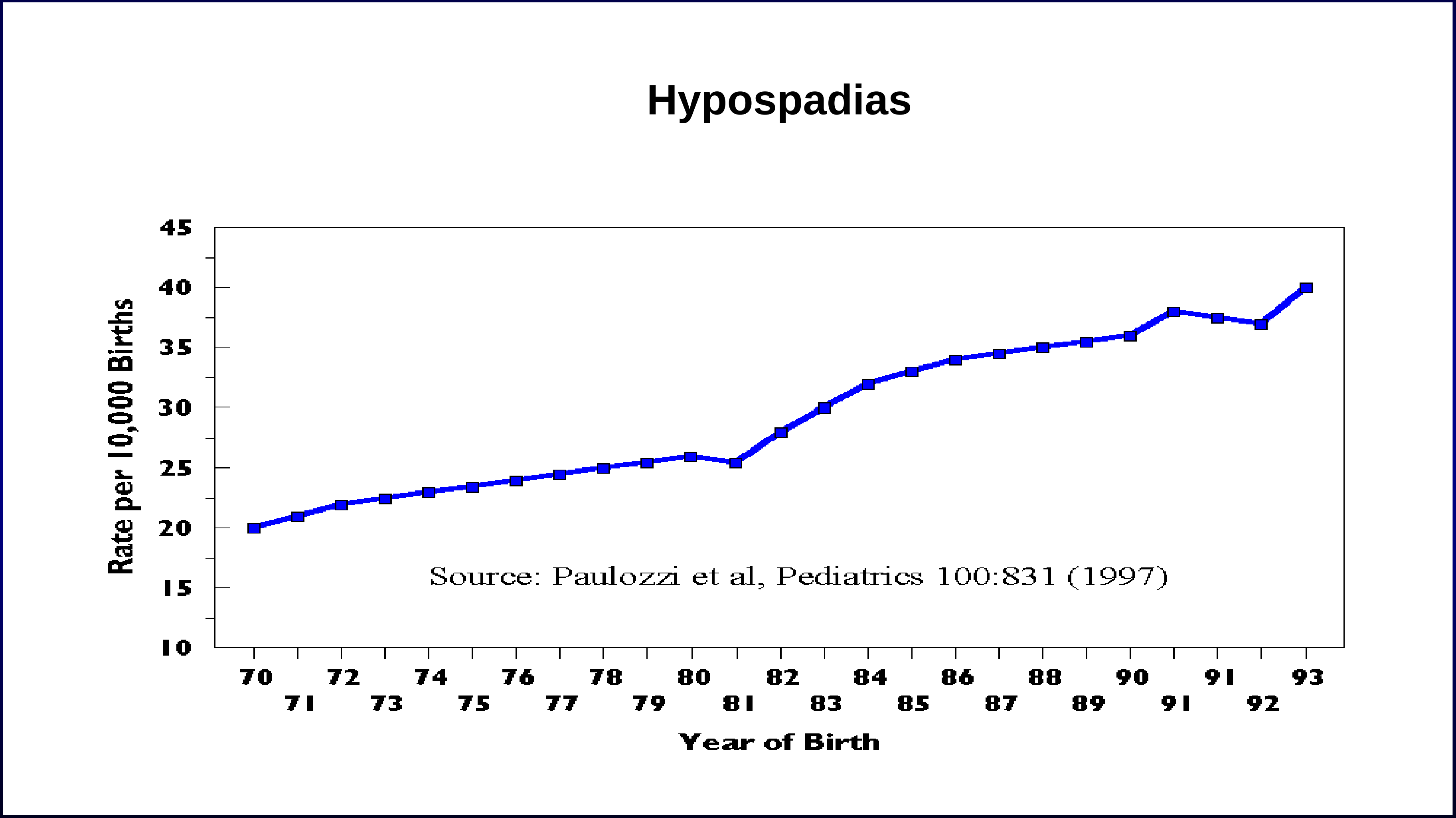
- Breast Cancer
- Endometriosis
- Shortened Lactation
- Declines in Male Reproductive Health
  - malformations, semen quality, cancer
- Altered Immune Function
- Developmental Learning Disabilities

Declining Reproductive Health



Carlsen et al, 1995 BMJ 305:609

Increased Malformations



PCBs and Human Development

- **Rice-Oil Poisonings**
  - **Japan, '68; Taiwan, '79**
  - **Yusho, Yu-cheng**
  - **4000 exposed**
    - **PCBs: 1 g, 40 ppm**
    - **PCDFs: 38 mg, 15 ppt**
  - **Perinatal Deaths**
  - **Low Birth Weights**
  - **Hyperpigmentation**
  - **Gum hypertrophy**
  - **Delayed Maturation**
  - **Cognitive Deficits**
- **Environmental Exposures**
  - **Four Cohorts**
    - **Fish Eaters (LM, EU)**
    - **Nursing Mothers (NC)**
    - **Gen. Population (NL, EU)**
  - **Motor Deficits**
    - hypotonia
  - **Memory Deficits**
  - **Immune Impairments**
  - **Thyroid Effects**
    - (TSH, T4)
  - **Consistent??**
  - **Transient??**
  - **Prenatal Critical Period**

Classes of EDCs

Effluents	BKME, STW
Flame Retardants	PBDEs
Fungicides	Vinclozolin
Herbicides	Atrazine
Insecticides	Methoxychlor
Metals	Tributyltin
Pharmaceuticals	Ethynyl Estradiol
Phenols	Bisphenol A
Plasticizers	Phthalates
Polyaromatic Hydrocarbons	PCBs, dioxin
Soy Products	Genistein
Surfactants	Alkylphenol Ethoxylates

Major EDC Uncertainties

- Exposure-Outcome Linkages
  - Latency
  - Persistent vs. non-persistent contaminants
  - Fate and transport
  - What effects are occurring in humans?
- Comparative toxicology
  - Sequence homology, binding, action
- Dose-response relationships
  - Shape, monotonicity
  - Interaction with “endogenous” diseases
  - Testing protocols

Major Uncertainties (Cont'd)

- Chemical diversity
  - ~100; Structures and potency; phytoestrogens
  - What will EDSP tell us?
- Multiple mechanisms of action
  - >1 receptor, co-factors and co-repressors
  - Dissimilar modes and similar phenotypes
  - Polymorphisms/Environmental Genome Project
- Cumulative exposures and effects
- Do EDCs need special consideration in risk assessment?

Summary

Is human health at risk?

- Data is present in animals
- Potential is present in humans
- Evidence in humans is controversial
- Dose (exposure)
- Homology
- Time (Sensitive periods)

FOOD QUALITY PROTECTION ACT

1996

Endocrine Disruptor Screening Program

- EPA Must
  - Screen pesticides for estrogenic effects that may affect human health
  - Develop a screening program
  - Report to Congress by August 2000

- Presently implementing the program
  - priority setting
  - validation of Tier 1 and Tier 2 assays

Framework

- Initial Sorting
- Priority Setting
- Screening (Tier 1)
  - Identifies substances for further testing
- Testing (Tier 2)
  - Identifies adverse effects and establishes dose-response relationship for hazard assessment

PROPOSED SCREENING BATTERY (Tier 1)

- *In vitro* Screens
  - ER Binding / Reporter Gene Assay*
  - AR Binding / Reporter Gene Assay*
  - Steroidogenesis Assay with minced testis
- In vivo Screens
  - Rodent 3-day Uterotrophic Assay (sc)
  - Rodent 20-day Pubertal Female Assay with Thyroid
  - Rodent 5-7 day Hershberger Assay
  - Frog Metamorphosis Assay
  - Fish Reproduction Screening Assay

* These assays are in the HTPS

ALTERNATE SCREENING ASSAYS

- Rodent 20-day Pubertal Male Assay with Thyroid
- Placental aromatase
- Rodent in utero through Lactation Assay

Endocrine Disruptor Screening Program

Tier 1 assays have the necessary breadth and depth to detect all currently known chemicals that may affect the endocrine, androgen and thyroid systems. Therefore, after having gone through the Tier 1 Screening battery, a chemical will be designated as having either:

- the potential for estrogen, androgen or thyroid activity, which will require further analysis
- in Tier 2 tests to verify and evaluate that potential; or
- low or no potential for estrogen, androgen or thyroid activity, which will allow the chemical to be put on "Hold."

Progress on Mammalian Tests

- Uterotrophic
- Hershberger
- Male and Female Pubertal
- In utero/lactational

Summary

Endocrine Disruptor Screening Program

- Methodical evaluation of chemicals
- Tier 1 test will identify most EDCs in vitro and in vivo
- Tier 1 identifies mode of action
- Tier 2 test will evaluate potency and risk to human

EPA's Endocrine Disruptors Screening Program: Legislation, Implementation, and Research

Elaine Z. Francis, Ph.D.
National Program Director for Endocrine Disruptors Research

Workshop on Effective Risk Management of Endocrine Disrupting Chemicals

January 29, 2001

EPA

Outline

- Regulations and Recommendations
  - FQPA and SDWAA
  - EDSTAC
- Implementation
  - Setting Priorities
  - Proposed Screening and Testing
  - Standardization and Validation
- Research
  - Supporting EPA’s screening & testing program
  - Overview of other EPA research
- Summary

Screening and Testing Program

EPA's Legislative Mandates (August 1996)

- Food Quality Protection Act
- Safe Drinking Water Act

Food Quality Protection Act Mandates

- EPA must:
  - Screen pesticides for estrogenic effects that may affect human health
  - Develop a screening and testing program by August 1998
  - Implement the screening and testing program by August 1999
  - Report to Congress by August 2000

FQPA - SDWA Discretionary Authority

- EPA can require screening and testing of:
  - Any pesticide active or inert ingredient
  - Any chemical that has an effect cumulative to an effect of a pesticide
  - Drinking water source contaminants
  - Other endocrine effects
  - Environmental effects (TSCA, FIFRA)

Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC)

- Chartered Oct 16, 1996 (www.epa.gov/scipoly/oscpendo)
- 39 members representing broad constituencies
- Advise EPA on:
  - A strategy for selecting and prioritizing chemicals for screening and testing
  - A process for identifying new and existing screening assays
  - A set of available screens for early application
  - What tests and when to test beyond screening
  - A process for identifying mechanisms for validation of screens and tests

Scope of Screening & Testing Program

- Based on current science and existing EPA testing authorities, EDSTAC did not limit the screening program to FQPA and SDWA
  - Estrogen, Androgen and Thyroid hormones
  - Human health and ecological effects
  - Expanded universe of chemicals and mixtures

The Universe of Chemicals

- Chemicals Under EPA's Purview
  - 900 pesticide active ingredients
  - 2500 "inerts" in 20,500 products
  - 75,500 industrial chemicals on the TSCA inventory
  - Environmental contaminants (?)
- Chemicals Under Others' Purview
  - Cosmetics
  - Food additives
  - Nutritional supplements

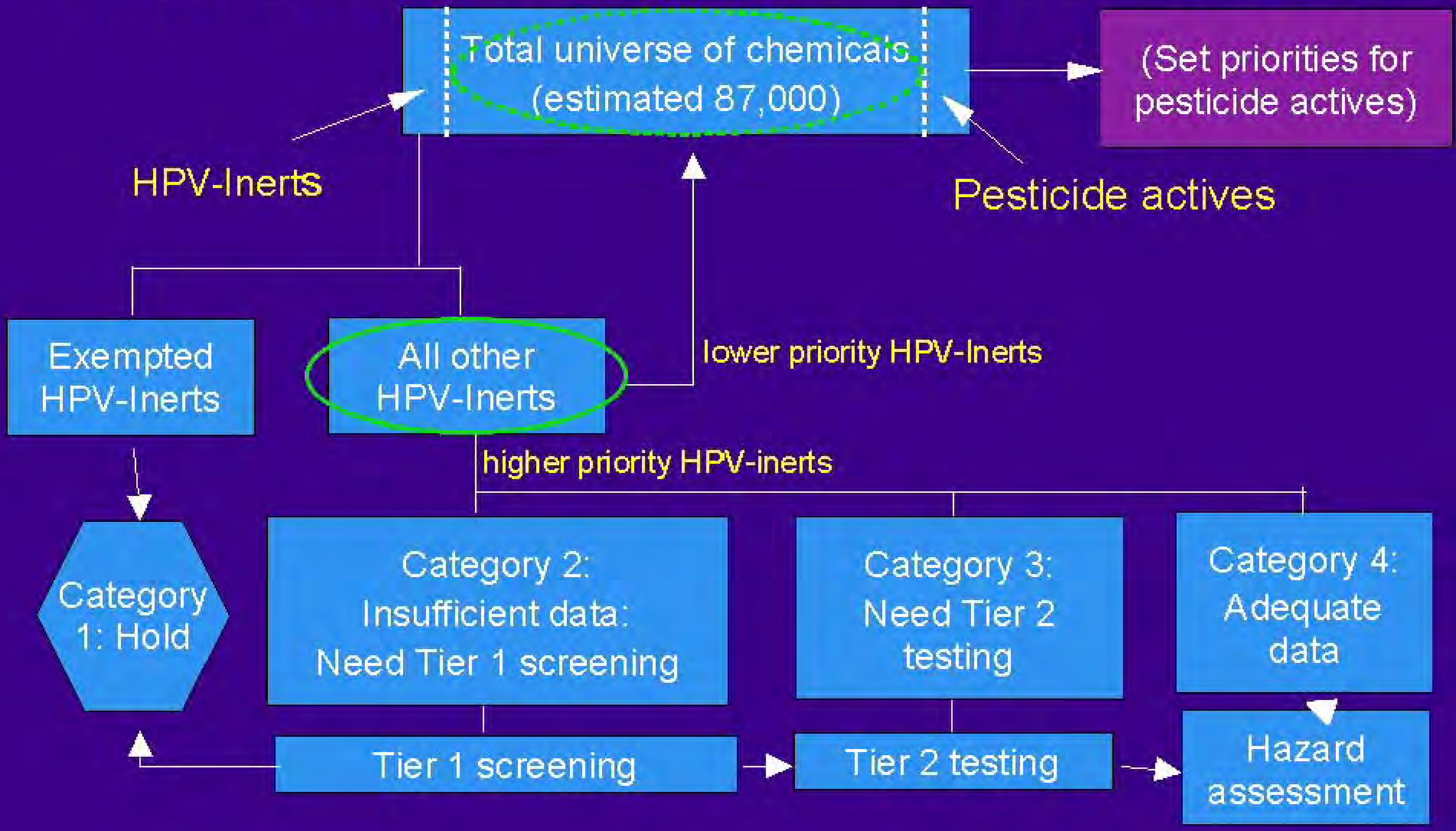
Framework

- Initial Sorting
- Priority Setting
- Screening (Tier 1)
  - Identifies substances for further testing
- Testing (Tier 2)
  - Identifies adverse effects and establishes dose-response relationship for hazard assessment

Proposed Phase I Strategy

- Restrict consideration of Phase I chemicals to pesticide active ingredients (~950) and chemicals which are both "inert" formulation ingredients and HPV commercial (~600) chemicals
- Meets statutory mandate for screening pesticides
- Robust statutory authority for obtaining data
- Reasonable size group of chemical candidates to focus on (1500-1600)

Current EDSP schematic for Phase I



Implementation Strategy: Commercial Chemicals

- **Goal:** Set priorities for chemicals according to potential exposure and potential endocrine effects
- **How:**
  - Develop Priority Setting Database
  - Group chemicals based on common effects and exposure information
  - Compare chemicals within groups and establish priorities within groups
  - Pick highest priority chemicals in each group

Default Scenario

File Edit What If Chemicals Reports Help

Specially Targeted Priorities

Exposure-Related Information

- Human Biological Monitoring Data
- Ecological Biological Monitoring Data
- Chemicals in Food and Drinking Water
- Chemicals in Consumer/Cosmetic Products
- Occupational Exposure Chemicals
- Surface Water Monitoring Data
- Indoor Air Monitoring Data
- Sediments/Soil Monitoring Data
- Persistence
- Bioconcentration
- Environmental Releases
- Production/Import Volumes
- Superfund Data
- Outdoor Air Monitoring Data

Effects-Related Information

- Epidemiological and Clinical Data
- Reproductive/Developmental Toxicity
- Carcinogenicity
- Subchronic Toxicity
- Ecotoxicity
- Quantitative Structure Activity Relationships

Combined Exposure- and Effects-Related Informat

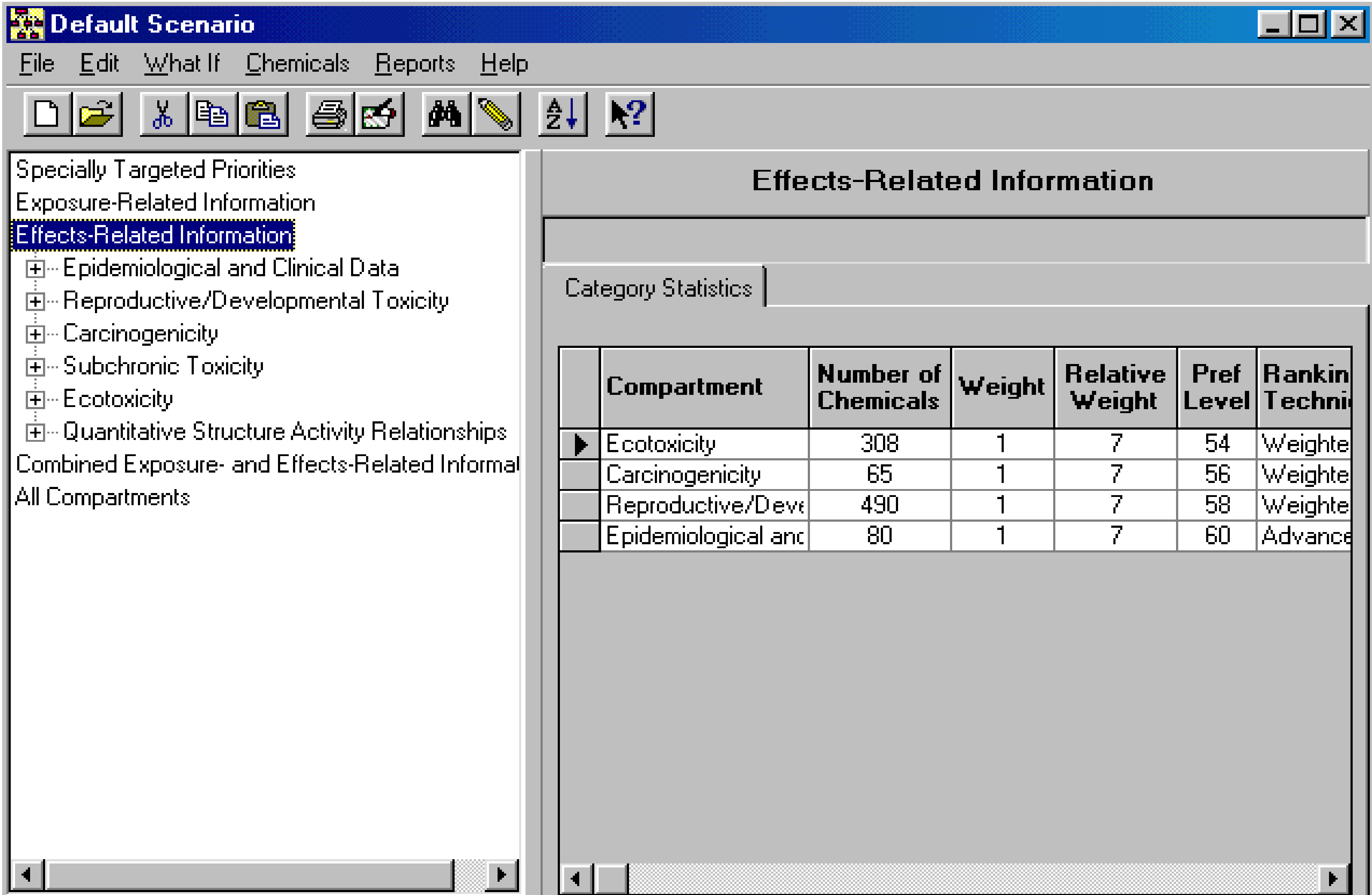
All Compartments

Exposure-Related Information

Category Statistics

Compartment	Number of Chemicals	Weight	Relative Weight	Pref Level	Rankin Techni
Persistence	103,435	0	0	50	Weighte
Sediments/Soil Mor	247	1	7	51	Weighte
Outdoor Air Monitor	363	1	7	52	Weighte
Superfund Data	419	1	7	53	Weighte
Chemicals in Food a	3,248	1	7	55	Weighte
Occupational Expo:	13,452	1	7	57	Weighte
Human Biological M	167	1	7	59	Weighte
Environmental Rele	442	0	0	61	Weighte
Bioconcentration	103,433	0	0	62	Weighte
Production/Import \	6,259	0	0	63	Weighte
Chemicals in Consu	3,162	1	7	64	Weighte
Indoor Air Monitorin	278	1	7	65	Weighte
Ecological Biologica	191	1	7	66	Weighte
Surface Water Mor	226	1	7	67	Advance

EDPSD v.2 Exposure Data Screen



EDPSD v.2 Effects Data Screen

Problem: Lack of Effects Information for Priority Setting

PROPOSED SOLUTION # 1

- Use High Throughput Screening Technology (HTPS)
- EPA to run ER, AR assays (Tier 1) assays before priority setting on 15,000 chemicals
- Use data to help set priorities for remainder of Tier 1 screen

PROPOSED SOLUTION # 2

- Develop and validate QSAR models based on receptor binding data

QSAR Models for Priority Setting

- Currently investigating 2 approaches
- Both address receptor binding
- Both need further validation
- Will be a data source for EDPSD
- Will be continually improved using data generated by the Screening Program

Proposed Tier 1 Screening Battery

- *In vitro* Screens
  - ER Binding / Reporter Gene Assay
  - AR Binding / Reporter Gene Assay
  - Steroidogenesis Assay with minced testis
- *In vivo* Screens
  - Rodent 3-day Uterotrophic Assay (sc)
  - Rodent 20-day Pubertal Female Assay with Thyroid
  - Rodent 5-7 day Hershberger Assay
  - Frog Metamorphosis Assay
  - Fish Reproduction Screening Assay

Alternate Screening Assays

- Rodent 20-day Pubertal Male Assay with Thyroid
- Placental aromatase
- Rodent *in utero* through Lactation Assay

Proposed Tier 2 Testing Battery

- Multigeneration reproduction and development studies
  - Mammals
  - Avians
  - Amphibians
  - Fish
  - Invertebrates

Research to Support EDSP: Overview

- Research contributions to protocol development
  - Receptor binding/transcriptional activation assays (<u>in vitro</u> screens
  - Tissue slice assay (*in vitro* screen)
  - Hershberger assay (*in vivo* screen)
  - Female pubertal assay in rats (*in vivo* screen)
  - Male pubertal assay in rats (*in vivo* screen)
  - Developmental toxicity screen in rats (*in vivo* alternative screen)
  - Frog metamorphosis assay (*in vivo* screen)
  - Fish 21-day reproduction screen in the fathead minnow (*in vivo* screen)
  - Two generation mammalian reproduction study (*in vivo* test)
  - Invertebrate reproduction assay (*in vivo* test)
- Developing position paper/case study

Protocol Development / Standardization / Validation

- ORD - conducting research, developing protocols, serving as a consultant on the standardization/validation
- OPPTS - the lead on the standardization/ validation
  - Standardization & demonstration of protocol
  - Validation in multiple laboratories
  - Scientific peer review
- EDMVS - advises EPA on standardization and validation issues
- ICCVAM - interagency coordinating committee on validating alternative methods

EPA Validation Process

- Method development and preparation of Detailed Review Paper (DRP)
- Pre-validation
  - Demonstration of relevance
  - Development of standardized protocol
- Determination of readiness for validation in consultation with EDVMS and ICCVAM
- Validation in multiple laboratories
- Independent peer review and review by ICCVAM

EPA Regulatory Implementation

- Use Order (FIFRA, FQPA, SDWA) and /or Test Rule authority (TSCA) to require industry to develop screening and testing data
- EPA conducts hazard/risk assessment

Endocrine Disruptors Will Be Regulated Under Existing Laws

- Insecticide, Fungicide, Rodenticide Act
- Toxic Substances Control Act
- Clean Water Act
- Safe Drinking Water Act
- Clean Air Act

Timeline

2000 2001 2002 2003 2004 2005

Priority Setting

Tier I Validation

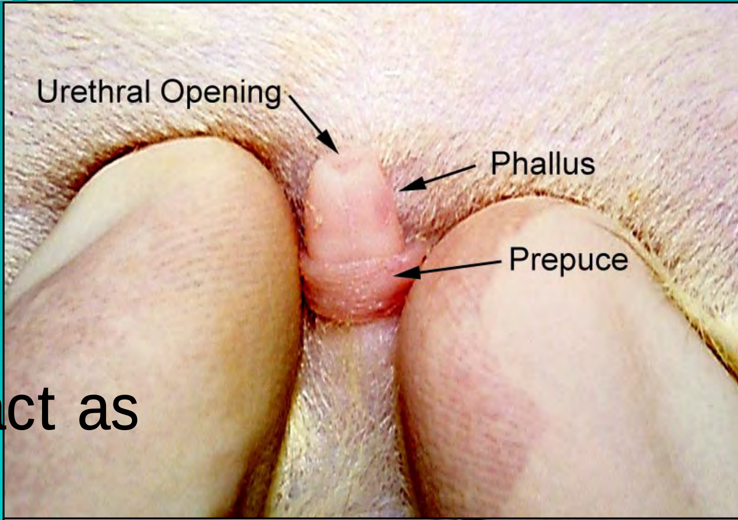
Tier II Validation

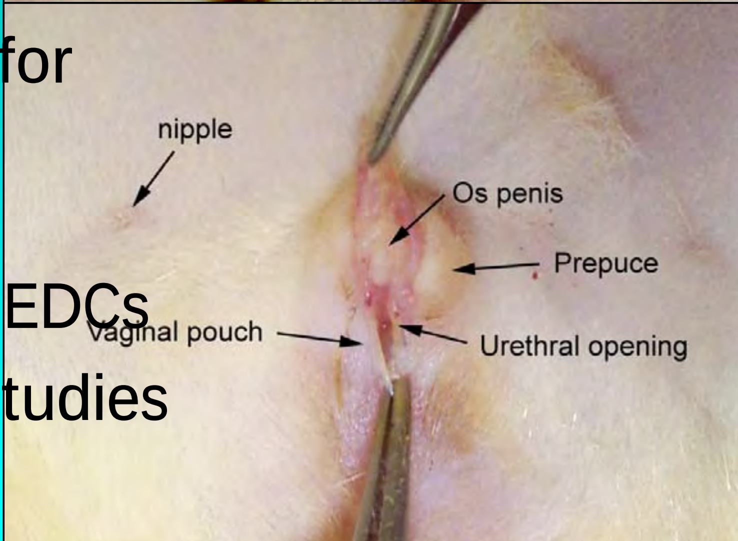
Phase I Implementation

Other EPA Research: Overview

Effects Research: LTGs 1, 2, & 3

- Evaluates adequacy of current testing guidelines
- Develops new/improved protocols for screening and testing program
- Determines classes of chemicals that act as EDCs and their potencies
- Determines the dose-response curves for EDCs at environmentally relevant concentrations
- Investigates mode of action of certain EDCs
- Conducts comparative endocrinology studies
- Examines population level effects





Exposure Research LTGs 1 & 2

- Identifies and improves understanding of major exposure routes and processes
- Develops predictive models estimating the extent and magnitude of exposures

Risk Assessment Research LTGs 1, 2 & 3

- Developing position paper on how results from EDSP could be incorporated into hazard characterization assessments
- Developing case study for methods on integrating human health and ecological EDC data into risk assessments

Risk Management Research - LTGs 1 & 2

- Identifying major sources of EDCs entering the environment, with focus on:
  - contaminated sediments
  - wastewater treatment plants
  - confined animal feeding operations
  - sources of combustion
  - drinking water treatment plants
- Developing tools for risk management of EDCs, such as biodegradation processes or pollution prevention strategies

Summary

Summary

- There is global concern regarding exposures to some environmental agents that interfere with endocrine systems
- Congressional mandates require that EPA establish a screening and testing program
- EPA is implementing such a program based on advisory committee recommendations that incorporates a tiered approach
- EPA's research is providing immediate results for implementing the screening and testing program

Summary (cont'd)

- EPA's long-term research program on EDCs focuses on the most critical uncertainties in determining whether humans and wildlife populations are being impacted by levels of EDCs in the environment, in identifying the sources of those exposures, and approaches to reduce/prevent them
- EPA is the only federal agency doing research in the area of risk management of endocrine disruptors

Monitoring Endocrine Disrupting Compounds(EDC's) in Aquatic Ecosystems in the U.S.

Steven L. Goodbred
U.S. Geological Survey
California State University
Sacramento, California

No Systematic Monitoring of just EDC's in the U.S.—Why?

- Incomplete consensus on EDC's
- Analytical methods still being developed for many compounds
- Difficulty selecting sampling matrix (sediment, water, tissue)
- Major funding needed

USGS

Several U.S. Agencies have National Monitoring Programs which include EDC’s

- U.S. Geological Survey
  (NAWQA, TOXICS, BEST)
- U.S. Environmental Protection Agency
  (EMAP, REMAP, National Dioxin Study)
- National Oceanic and Atmospheric Administration
  (Mussel Watch, Benthic Surveillance)
- Food and Drug Administration
  (National Monitoring Program for Food and Feed)

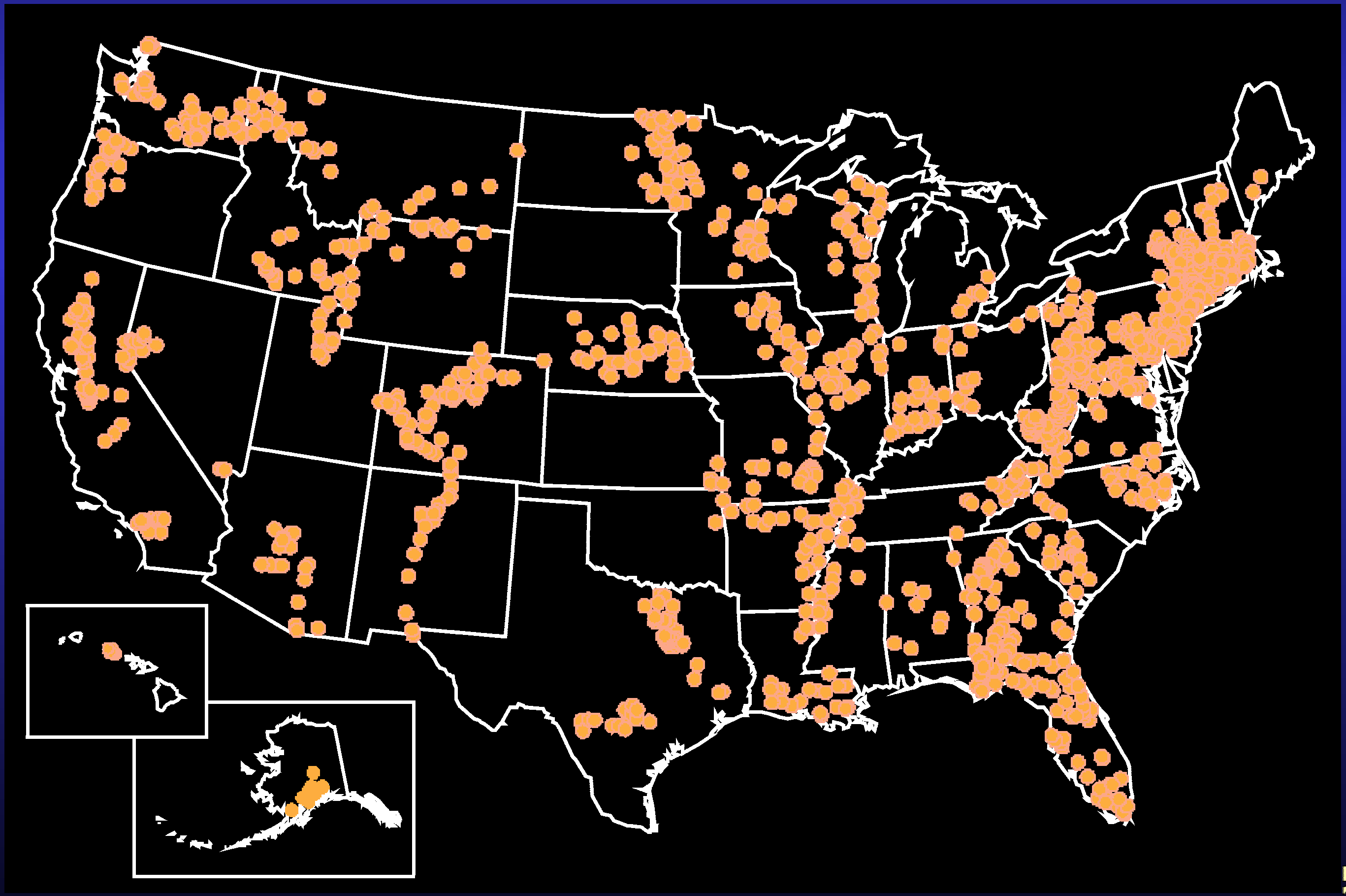
USGS

USGS National Water Quality Assessment Program(NAWQA) http://water.gov/NAWQA

- Established in 1991
- Assess status of trends of U.S. surface and ground water
- Elucidate factors that affect water quality
- Rigorous field and lab QA/QC
- Nationally consistent chemical and ecological data collection and analysis

USGS

Chemical Monitoring Sites in USGS's National Water Quality Assessment Program



USGS

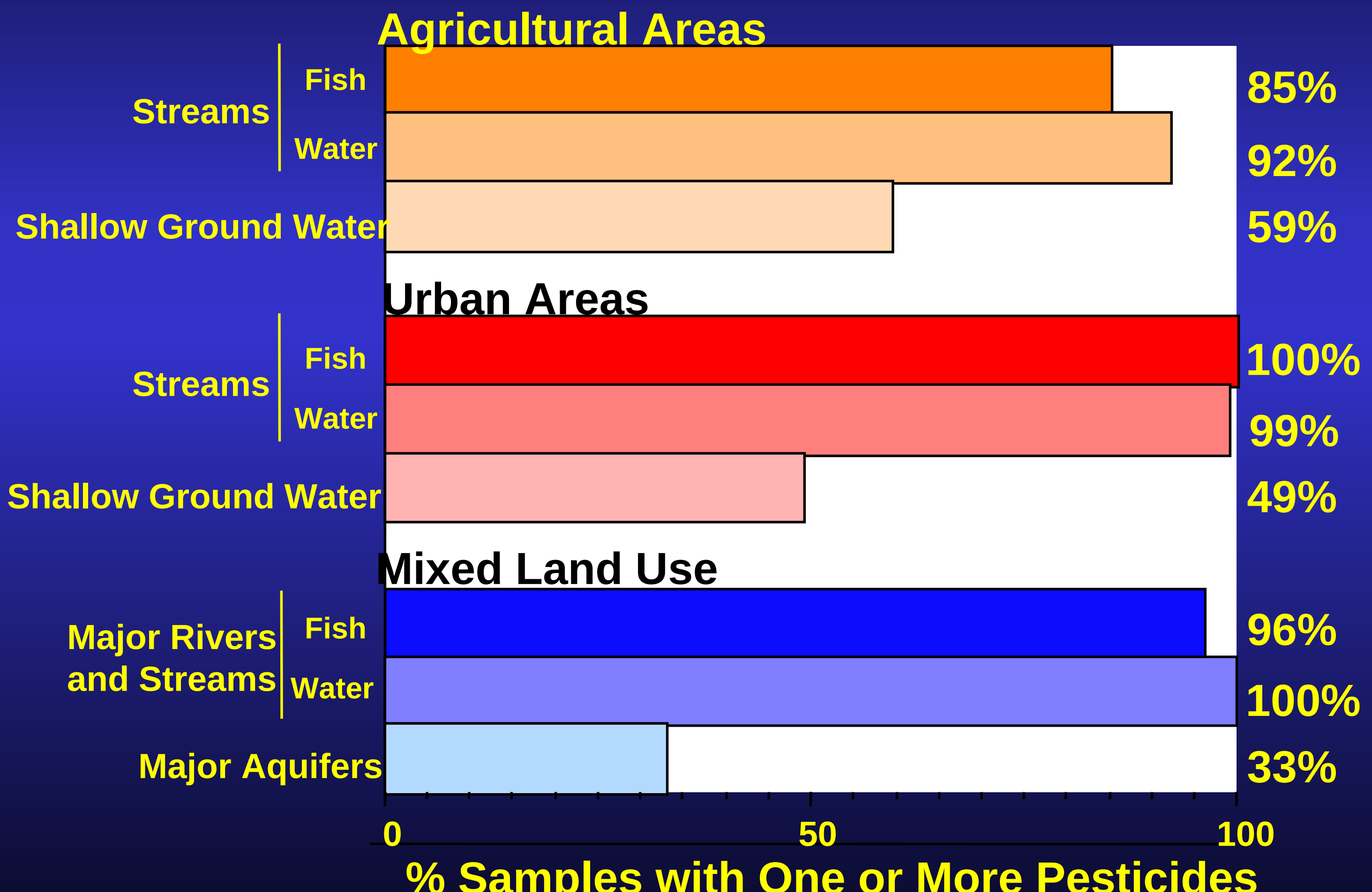
Organic Compounds Monitored in the U.S. by USGS, NAWQA

Matrix	# compounds	#EDC's *
Water (pesticides)	80	20
Bed Sediment (PAHs, phenols, etc.)	80	17
Fish Tissue (OCs, PCBs)	28	24
Total Suspected EDC's		44

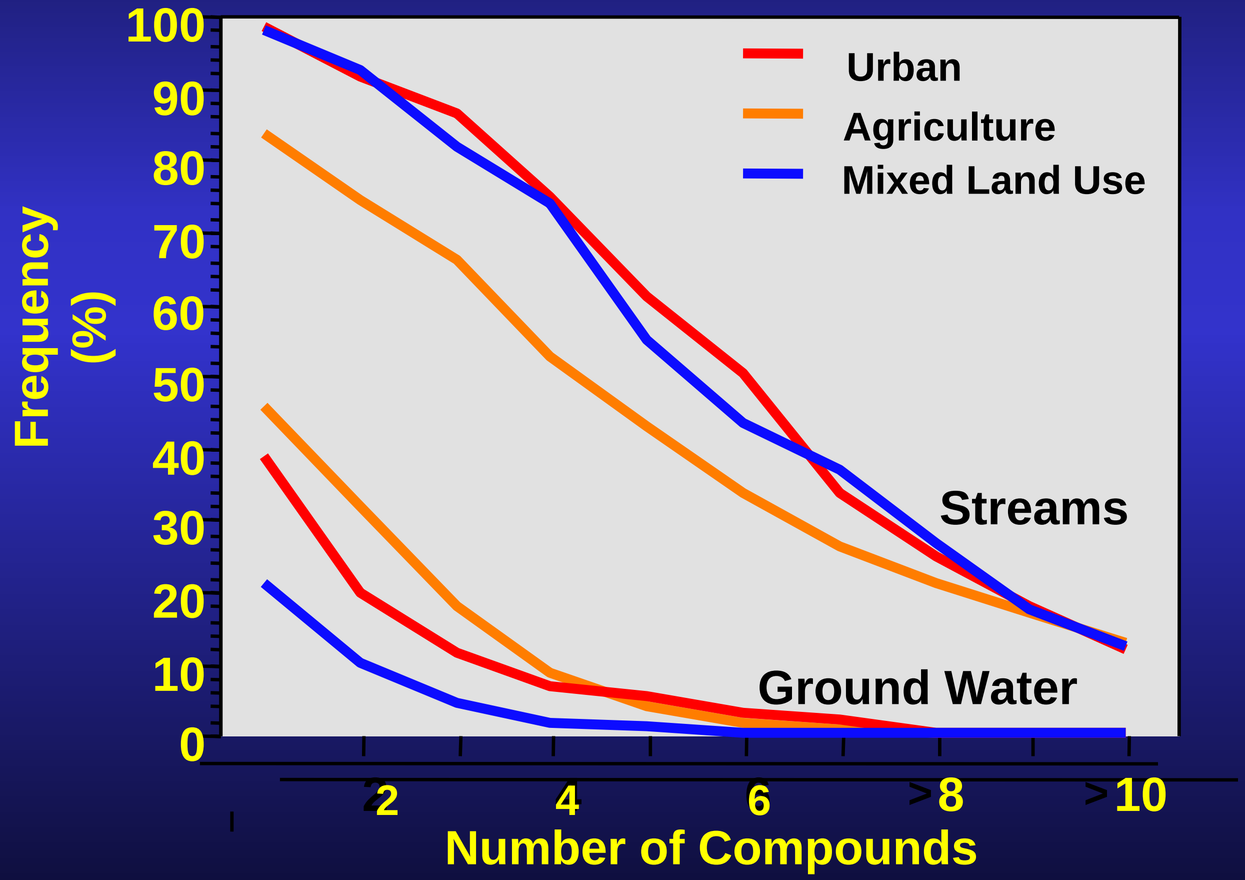
* from Keith, 1997

USGS

Pesticides widespread in streams & ground water

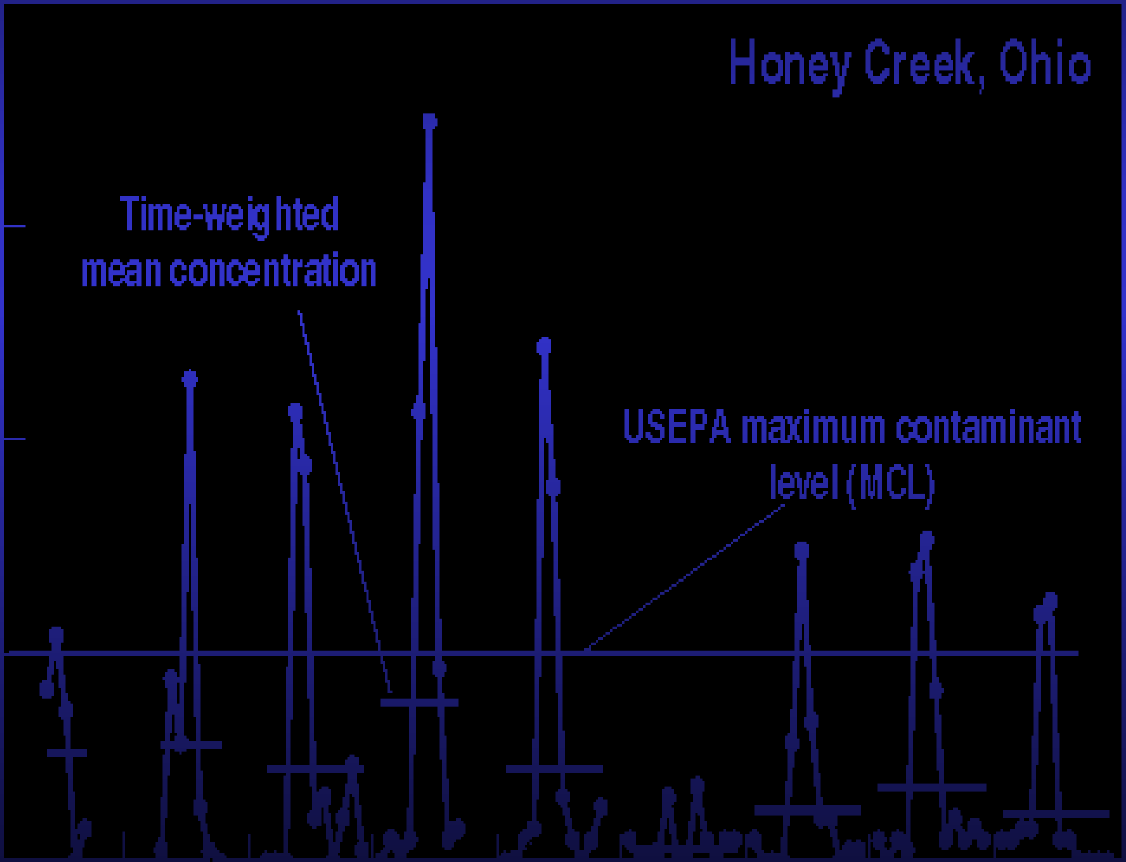


Pesticides almost always occur as mixtures



USGS

Pesticides in streams usually occur in strong seasonal pulses



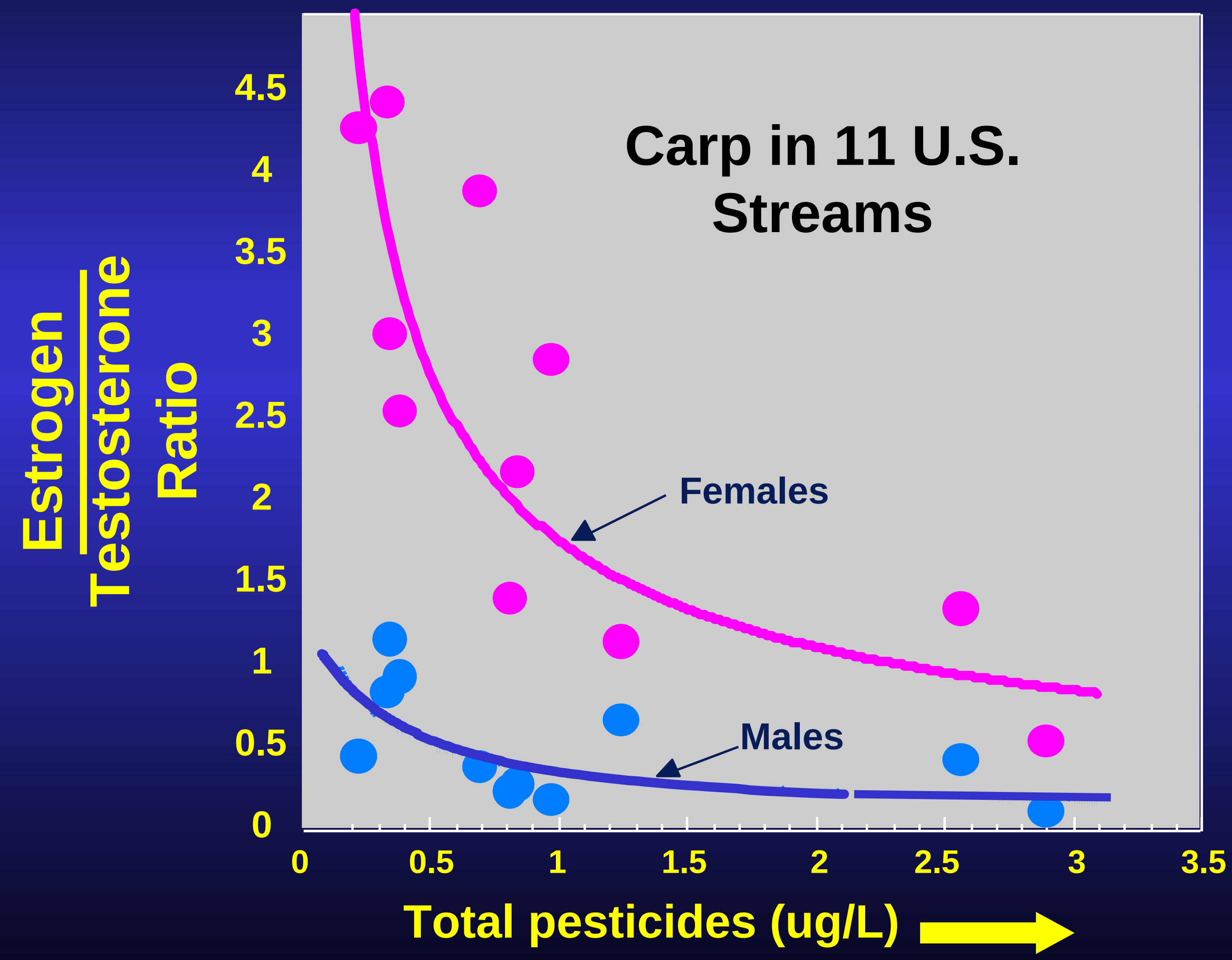
Breakdown products often total 10-25 times the concentration of parent compounds

Herbicide breakdown products

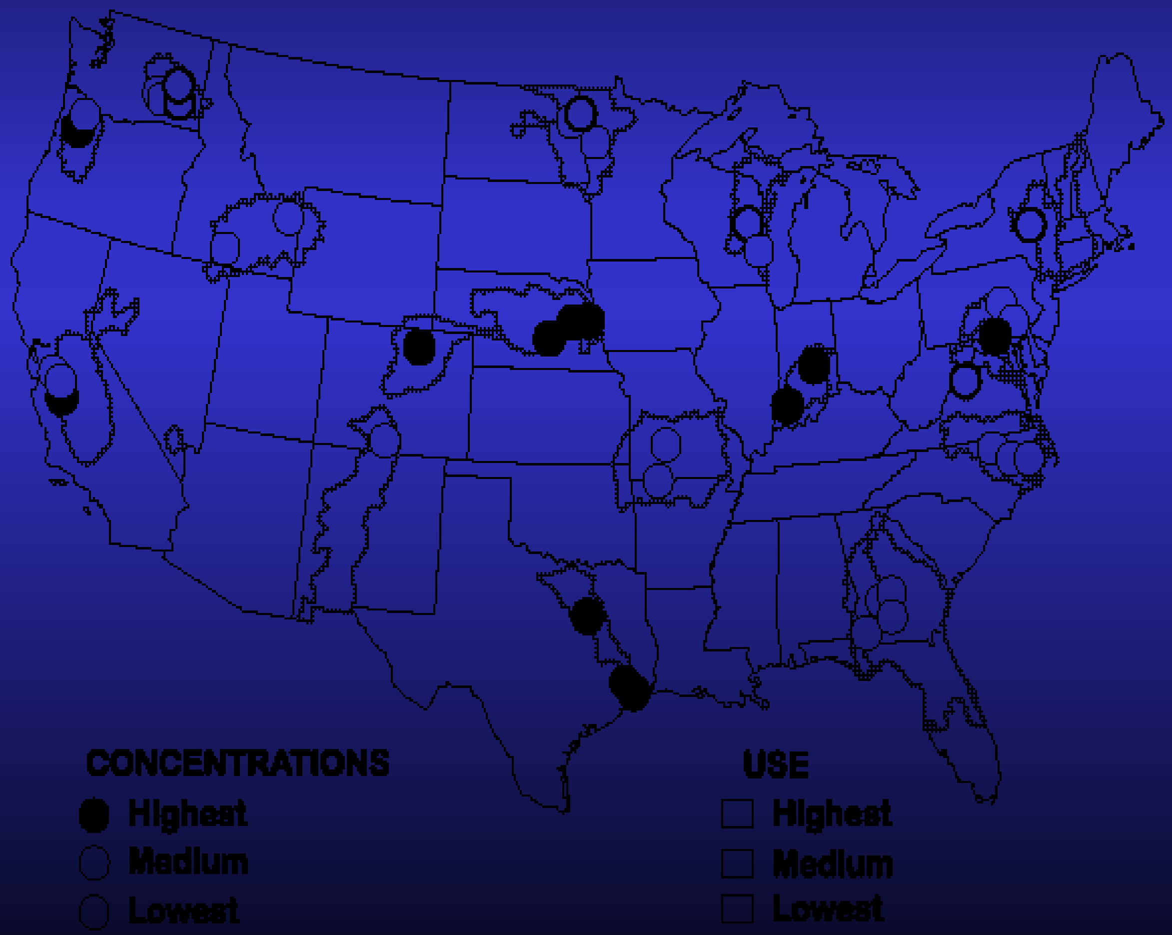
Concentration, ug/L

Herbicide parent compounds

Reconnaissance data show potential of pesticides to affect fish endocrine systems



Herbicides in agricultural streams were highest in the Corn Belt



Herbicides that are EDCs in U.S. Surface Water

Land use	# of samples	Detection frequency	Percentile (ug/L) 50%	Percentile (ug/L) 95%	Maximum (ug/L)
ATRAZINE					
Ag	1559	85%	.04	3.51	120
Urban	611	85%	.02	.36	14
SIMAZINE					
Ag	1560	59%	.01	.22	9
Urban	611	78%	.02	.70	4

USGS

Organochlorines and PCBs in Fish from the U.S.

Land use	# of samples	Detection frequency	Percentile (ug/kg WW) 50%	95%	Maximum (ug/kg WW)
P,P' DDE					
Ag	171	88%	43	3,080	7,300
Urban	60	90%	34	160	370
Forest	73	38%	<MDL	87	300
PCBs					
Ag	171	38%	<MDL	209	1,200
Urban	60	83%	150	1,850	72,000+
Forest	73	8%	<MDL	108	550

USGS

PAHs in Bed Sediment from the U.S.

Land use	# of samples	Detection frequency	Percentile (ug/kg WW) 50%	Percentile (ug/kg WW) 95%	Maximum (ug/kg)
PYRENE					
Ag	131	89%	410	4,840	11,000
Urban	219	21%	<MDL	381	4,200
Forest	112	26%	<MDL	648	2,200
PHENANTHRENE					
Ag	220	12%	<MDL	191	3,300
Urban	114	79%	185	3,770	10,000
Forest	116	18%	<MDL	334	1,900

USGS

What can we conclude about EDC's from NAWQA

- Several suspected EDC's widespread in U.S. streams, surface water, sediment and fish
- Occurrence follows land and chemical use
- Many EDC's at low levels(<ppb) so difficult to assess potential endocrine disruption
- Also complicated by exposure to chemical mixtures and pulses of pesticides
- Low levels of dissolved pesticides are associated with changes in hormone ratio

USGS

science for a changing world

A National Reconnaissance of Pharmaceuticals and other Emerging Contaminants in U.S. Streams

Toxic Substances Hydrology Program

Why Pharmaceuticals?

Project Goal

Determine if pharmaceuticals, antibiotics, hormones, and other wastewater related compounds are entering the environment through human, animal, and industrial wastewaters.

USGS

Approach

- Develop sensitive and specific analytical methods

- Evaluate environmental occurrence in “susceptible” waters

USGS

Target Chemicals (95 OWCs)

- 22 Antibiotics (lincomycin, trimethoprim)
- 14 Prescription Drugs (fluoxetine, gemfibrozil, ranitidine)
- 5 Nonprescription Drugs (caffeine)
- 15 Hormones and Sterols (equilin)
- 39 Household and Industrial Compounds (triclosan, bisphenol A)

USGS

Target Hormones and Sterols

Biogenic Hormones

- 17b-Estradiol
- 17a-Estradiol
- Estrone
- Estriol
- Testosterone
- Progesterone
- cis-Androsterone
- Equilenin *
- Equilin *

Synthetic Hormones**

- 17a-Ethynylestradiol
- Mestranol
- 19-Norethisterone

Sterols

- Cholesterol
- 3b-Coprostanol
- Stigmastanol
  (plant sterol)

* Hormone replacement therapy; commonly prescribed

** Ovulation inhibitor

USGS

Potential Sources

- WWTF
- On Site Septic Systems
- Animal Feeding Op.'s
- Industrial Discharges (Medical)

USGS

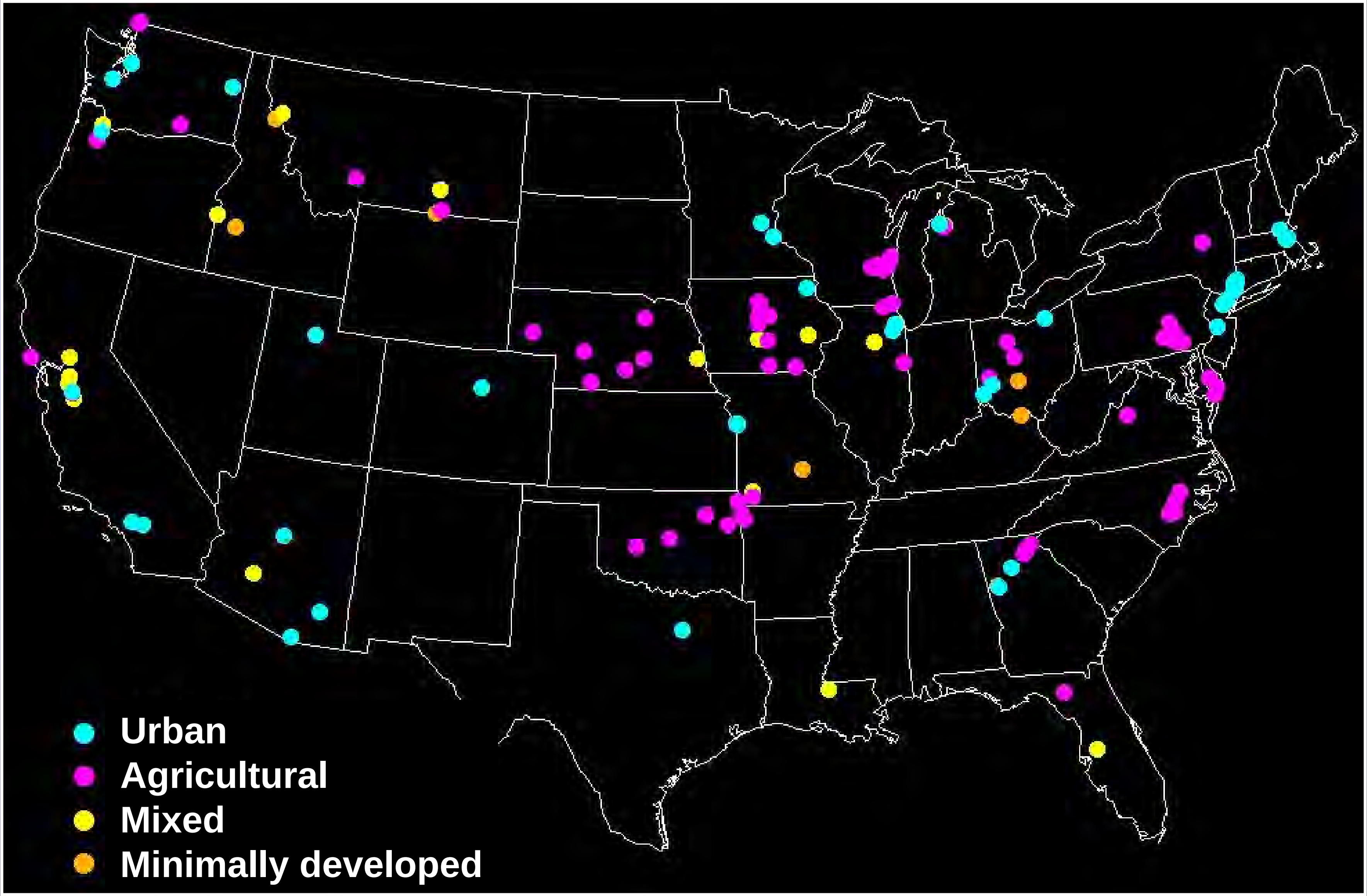
Phase I: Assessing Environmental Occurrence in Streams Susceptible to Sources of OWCs

139 streams in 30 states

- 62 Agricultural
- 52 Urban
- 17 Mixed Land Use
- 8 Minimally developed

USGS

Stream Monitoring Network (1999-2000)



USGS

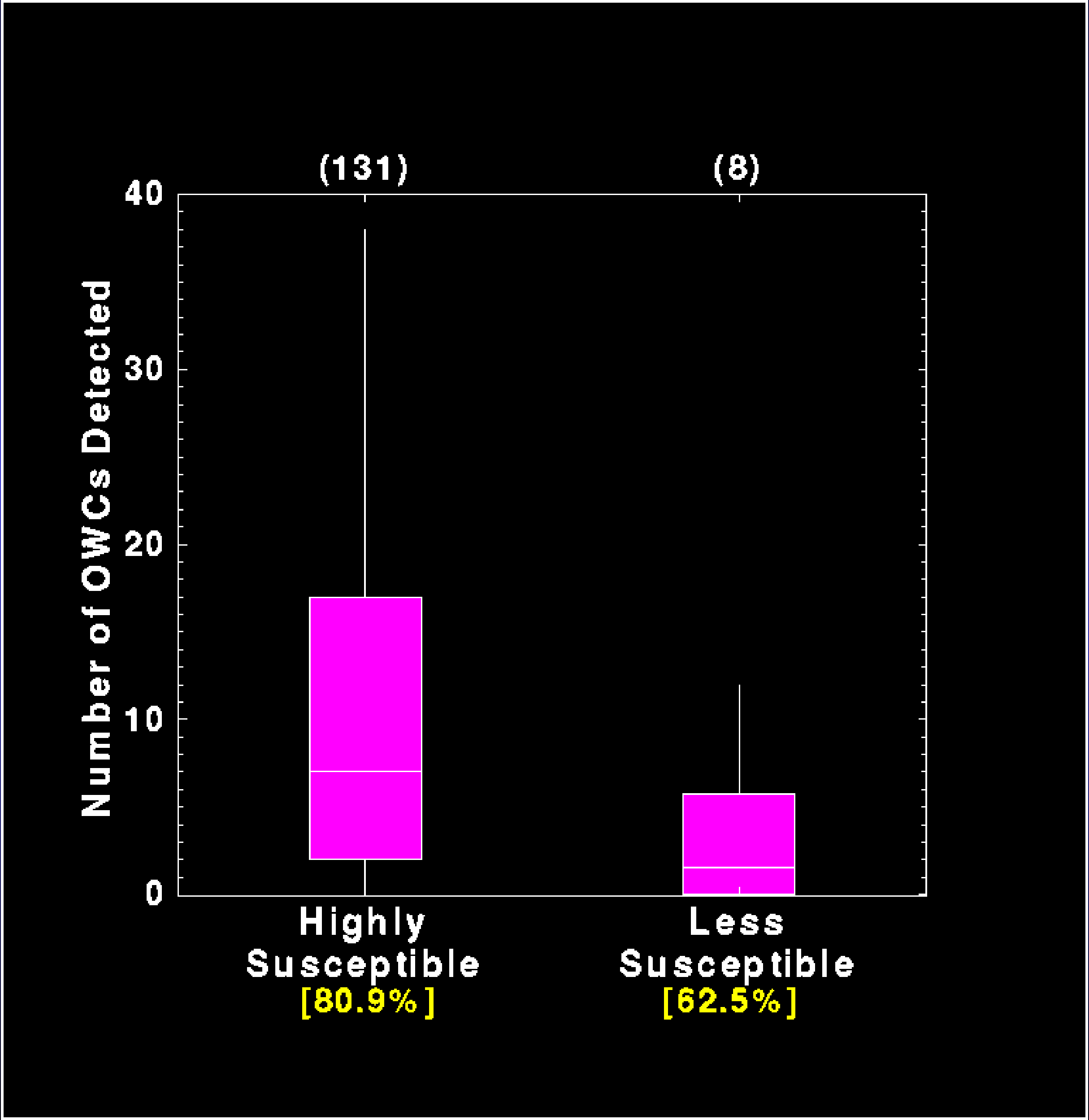

KANN

General Results

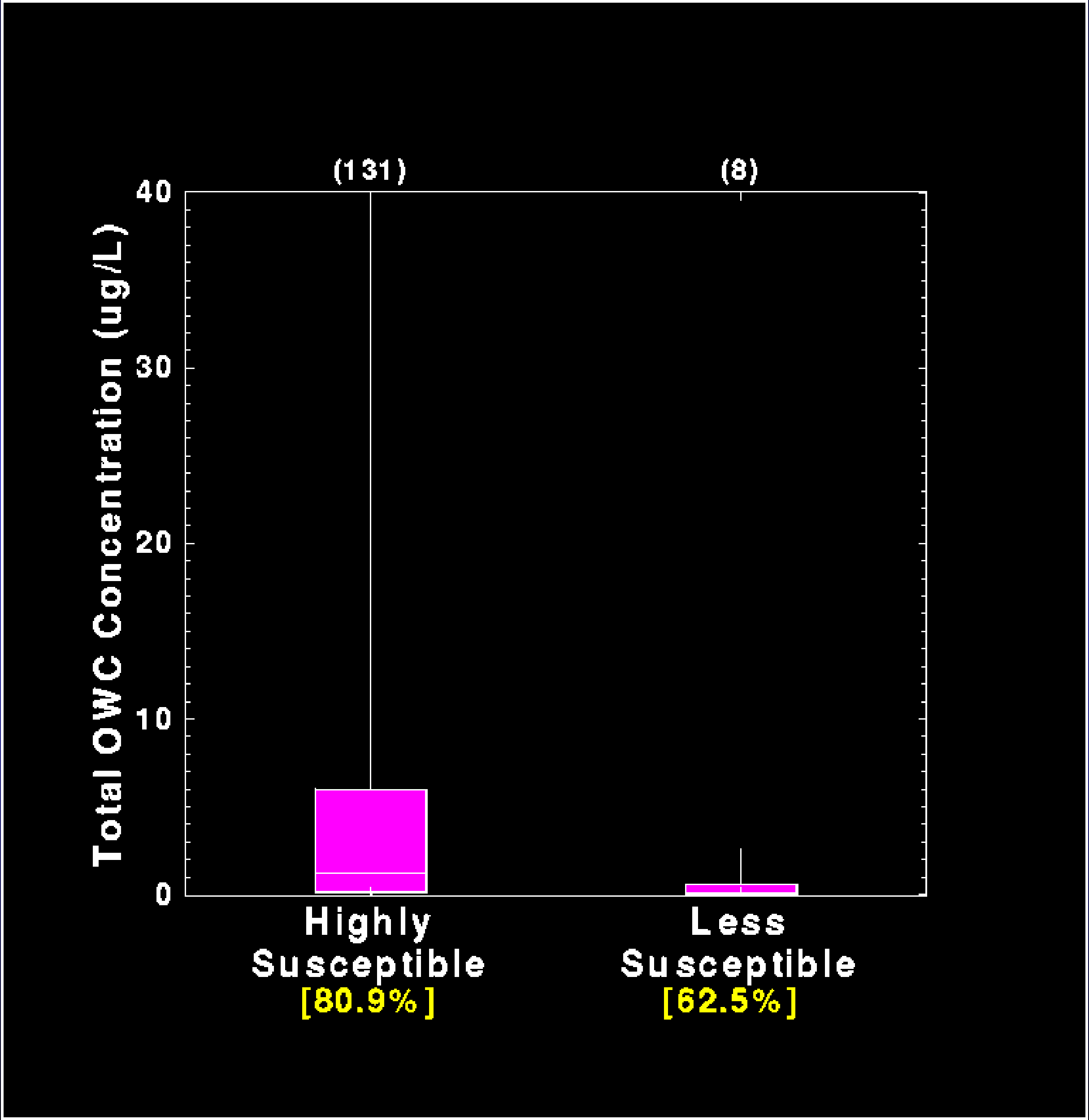
- OWCs found in most streams sampled (~80%)
- 82 of 95 OWCs detected, representing a wide range of uses and origins
- Measured OWCs concentrations generally low
  - few standards or guidelines exceeded (few exist)
- Detection of multiple OWCs common
  - about 75% had more than 1 OWC
  - about 35% had more than 10 OWCs

USGS

Number of OWCs Detected



Aggregate Concentrations

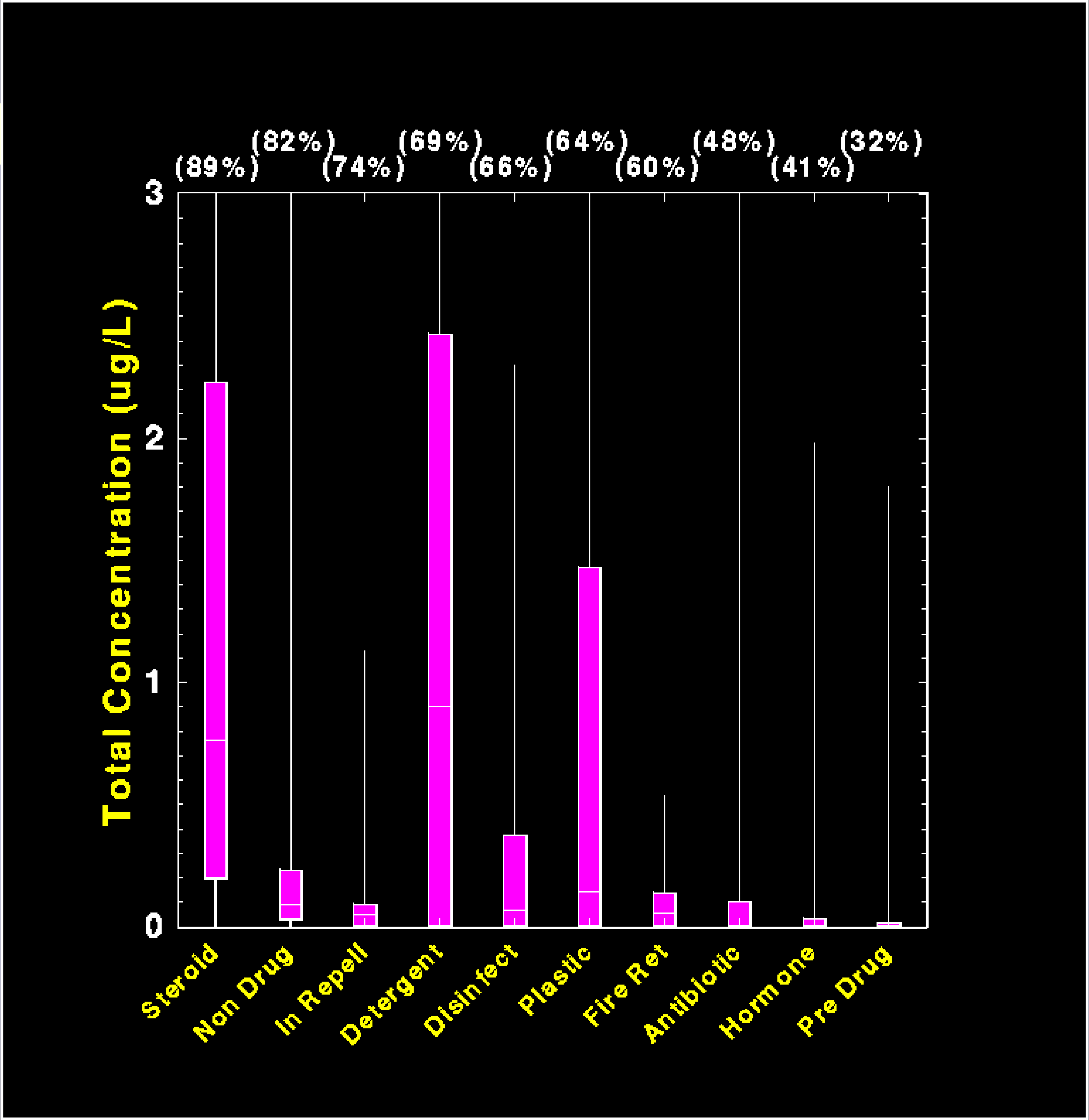


USGS

Compound Classes

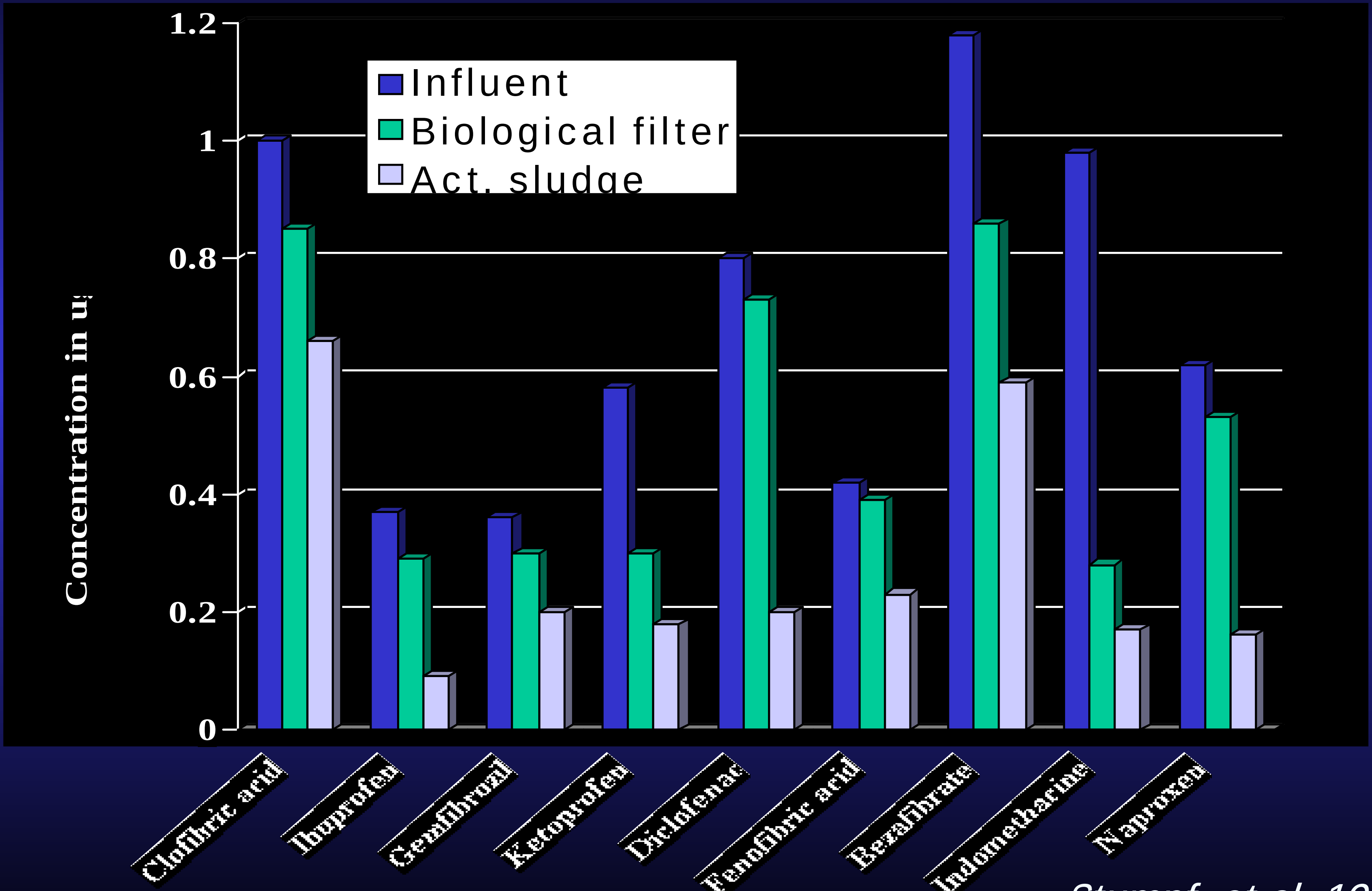
Compare:

- Detection Frequency
- Concentration



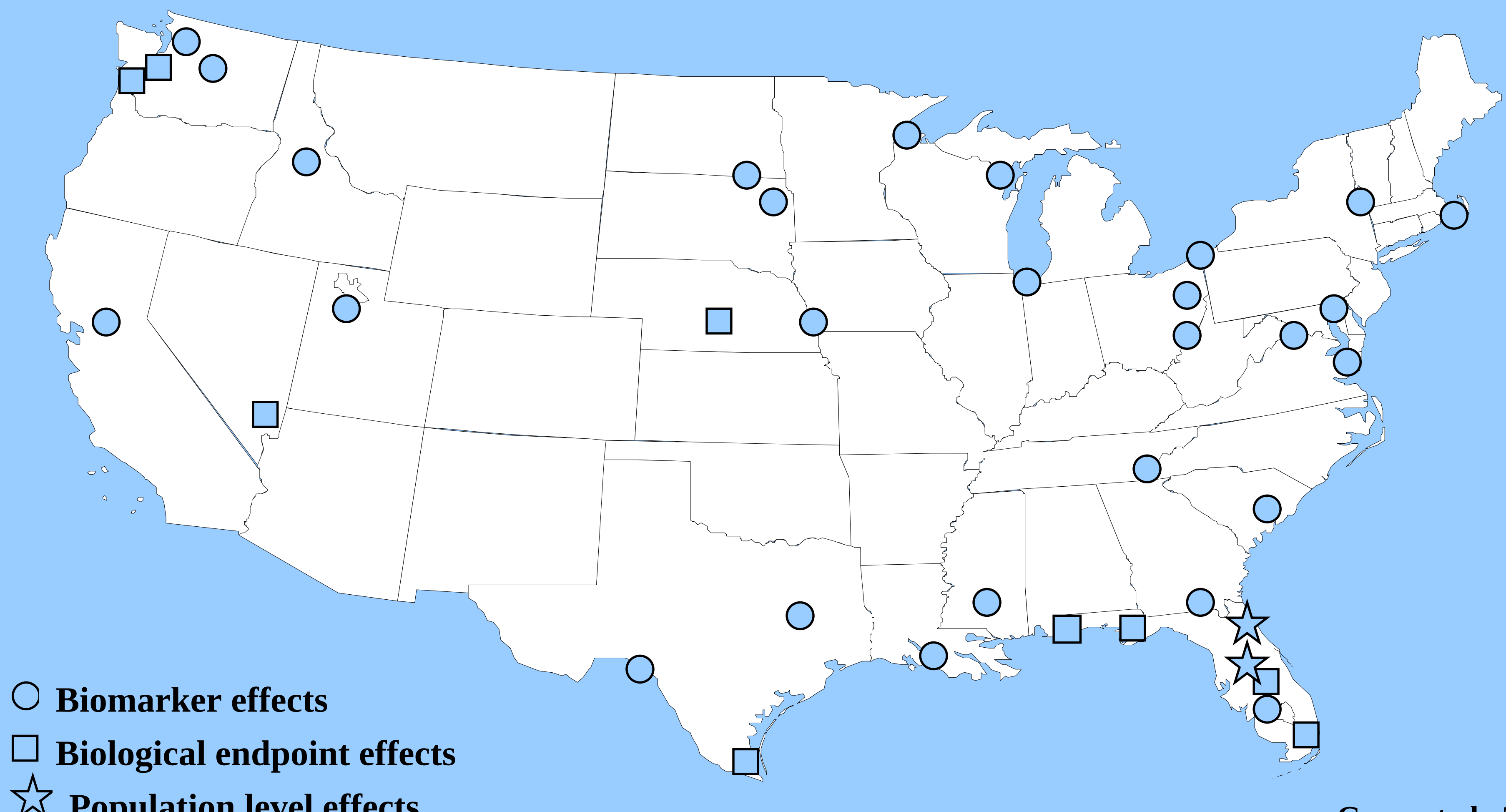
USGS

Removal through WW Treatment ?



Stumpf, et al, 1999

Is Endocrine Disruption Widespread? DOI Study Sites Showing Evidence of Endocrine Disruption in Wildlife



Gross et al. 2000

For More Info, Try the Internet

The National Reconnaissance:
toxics.usgs.gov/regional/emc.html

The Toxics Program:
toxics.usgs.gov

The USGS:
www.usgs.gov

USGS

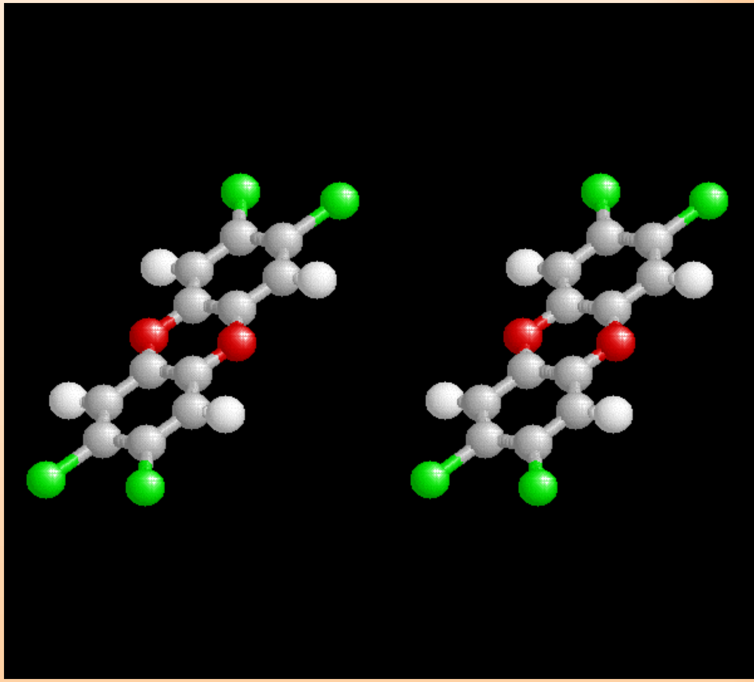
ENDOCRINE DISRUPTORS FROM COMBUSTION AND VEHICULAR EMISSIONS: IDENTIFICATION AND SOURCE NOMINATION

Brian Gullett[1*], Jeff Ryan[1], Paul Lemieux[1], Carolyn Acheson[2], Michael DeVito[3], James Rabinowitz[3], Sukh Sidhu[4], Richard Striebich[4], Joy Klosterman[4]

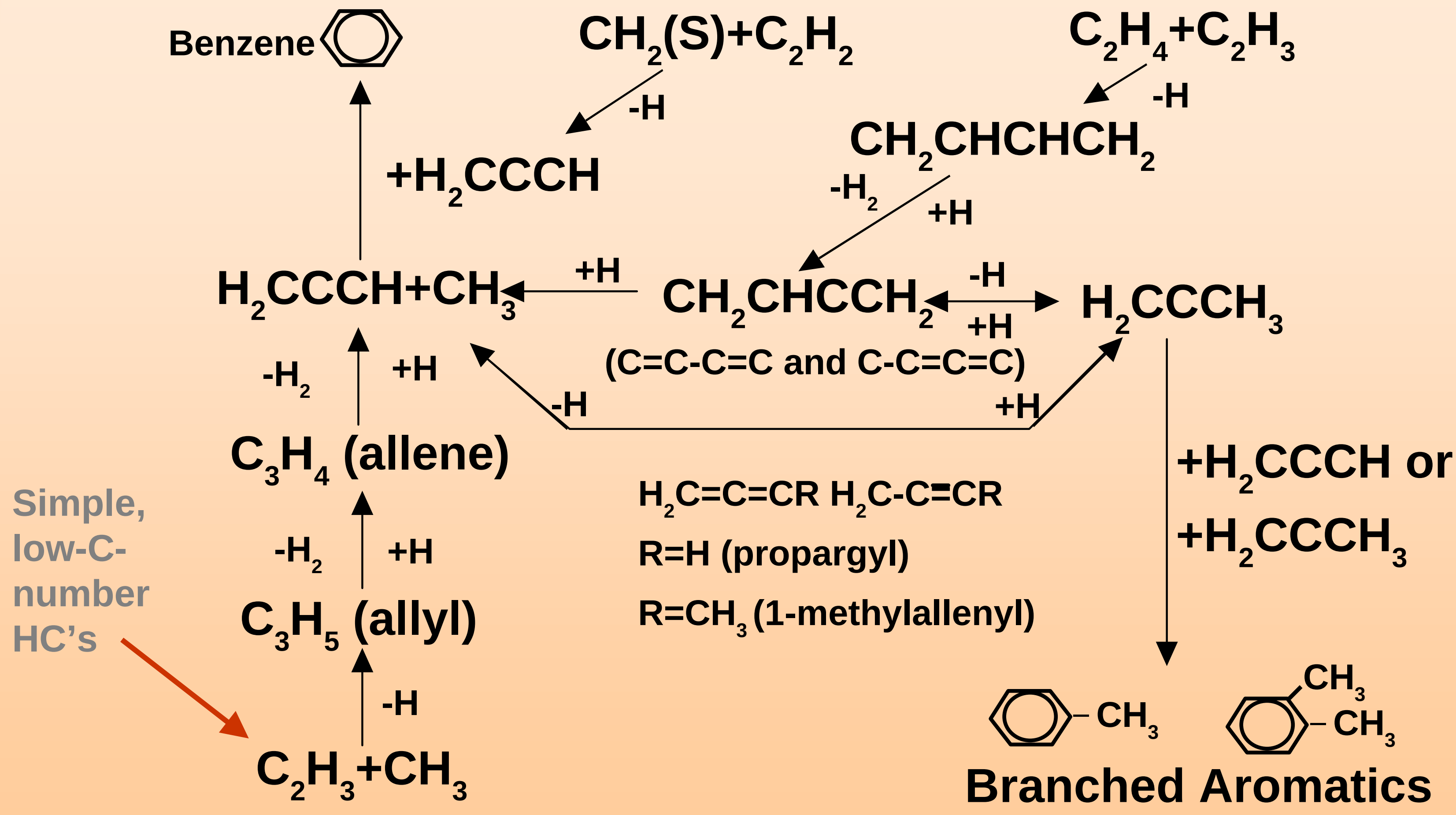
U.S. Environmental Protection Agency
[1]National Risk Management Research Laboratory, Air Pollution Prevention and Control Division (MD-65), Research Triangle Park, NC 27711
[2]National Risk Management Research Laboratory, Cincinnati, OH 45268
[3]National Health and Environmental Effects Research Laboratory, Toxicology Division, Research Triangle Park, NC 27711
[4]University of Dayton Research Institute, 300 College Park, Dayton, OH 45469
*919-541-1534, 919-541-0554 (fax), gullett.brian@epa.gov

BACKGROUND

- A significant fraction of organic emissions from combustion sources remains uncharacterized
- 100’s of compounds are emitted
  - e.g., PAHs, oxygenates, alkyl phenols
- We only see what we specifically look for
  - e.g., PCDD/Fs “dioxins”
- EDCs are emitted from combustion sources
  - e.g., PCDD/F, PCB, Hg
- Combustion sources are ubiquitous
  - constitute a major exposure source



GAS PHASE, AROMATIC PRECURSORS – How do we get complex aromatic structures?



After Marinov, Pitz, Westbrook, Castaldi and Senkan, 1996

OBJECTIVE

We will survey combustion sources for potential endocrine disruption activity, try to isolate the compounds responsible for such activity, and attempt to estimate their emission factors.

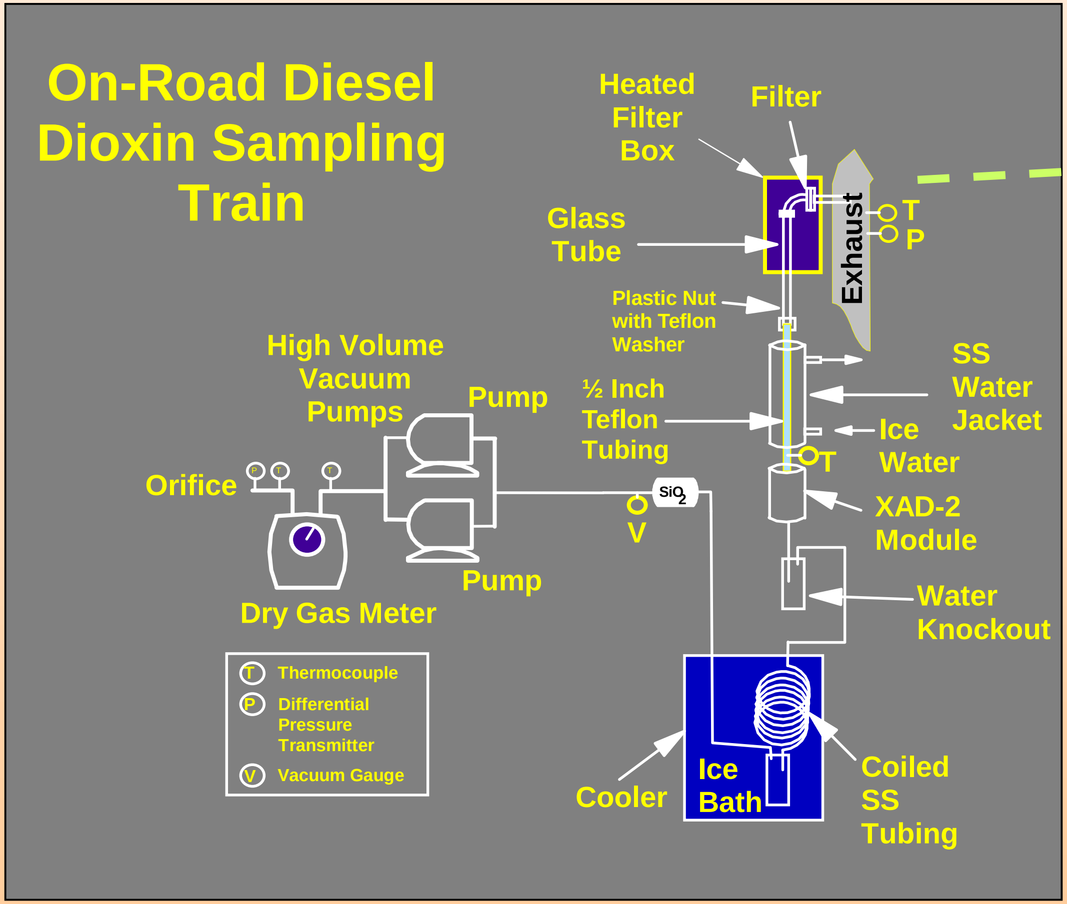
APPROACH

- Opportunistic combustion source sampling
  - domestic waste burning, diesel trucks (HDDVs), forest fires, fireplaces, and woodstove….others.
- Bioassays
  - Yeast estrogen assay
  - CALUX
  - Vitellogenin mRNA Assay
- Sample fractionation to isolate target compounds
  - HPLC technique (L. Brooks)
  - Capillary Electrophoresis
  - TIE method (G. Ankley)
- Multi Dimensional Gas Chromatography
  - diagram/description
- QSAR, Statistical analyses
  - Structure clues

Source Characterization

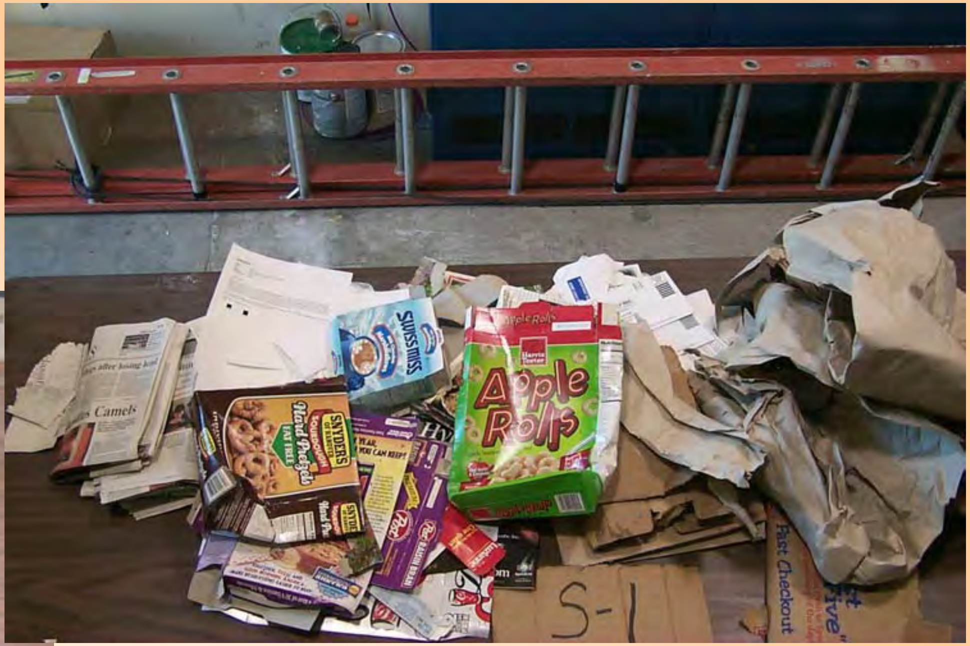
- Done
  - diesel truck
  - woodstove
  - sewage sludge incinerator
  - domestic waste
- Future
  - pine straw
  - structural fires
  - oil spill fire
  - municipal waste, full scale
  - DoD diesel truck

On-Road Diesel Sampling



“Uncontrolled” Burning Sources

Burning of Domestic Waste



Wheat Straw Testing

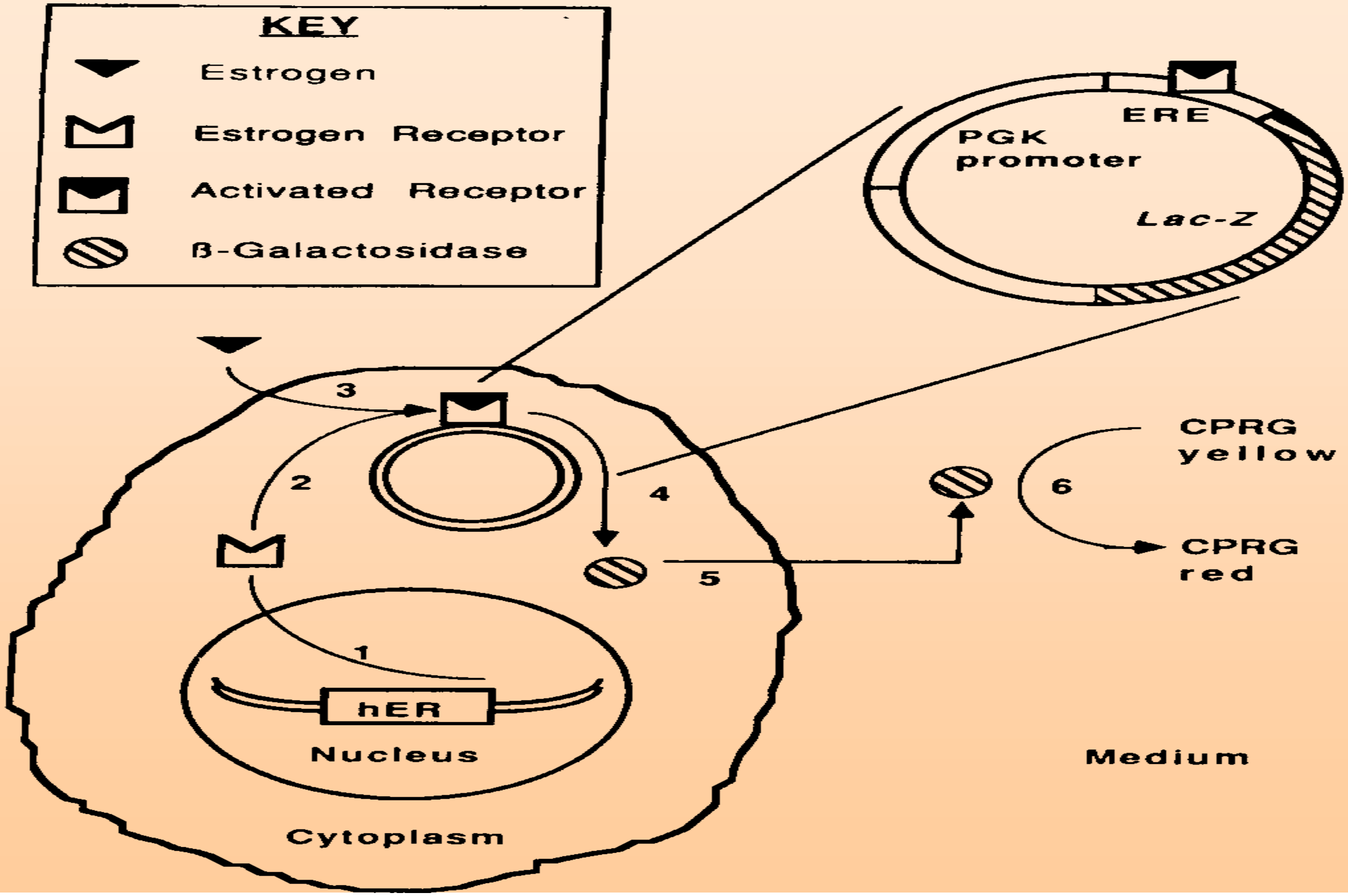
CA PCDD/F, PCB Sources?

FIREPLACE

WOODSTOVE

SAMPLE CHARACTERIZATION

Yeast Estrogen Screen

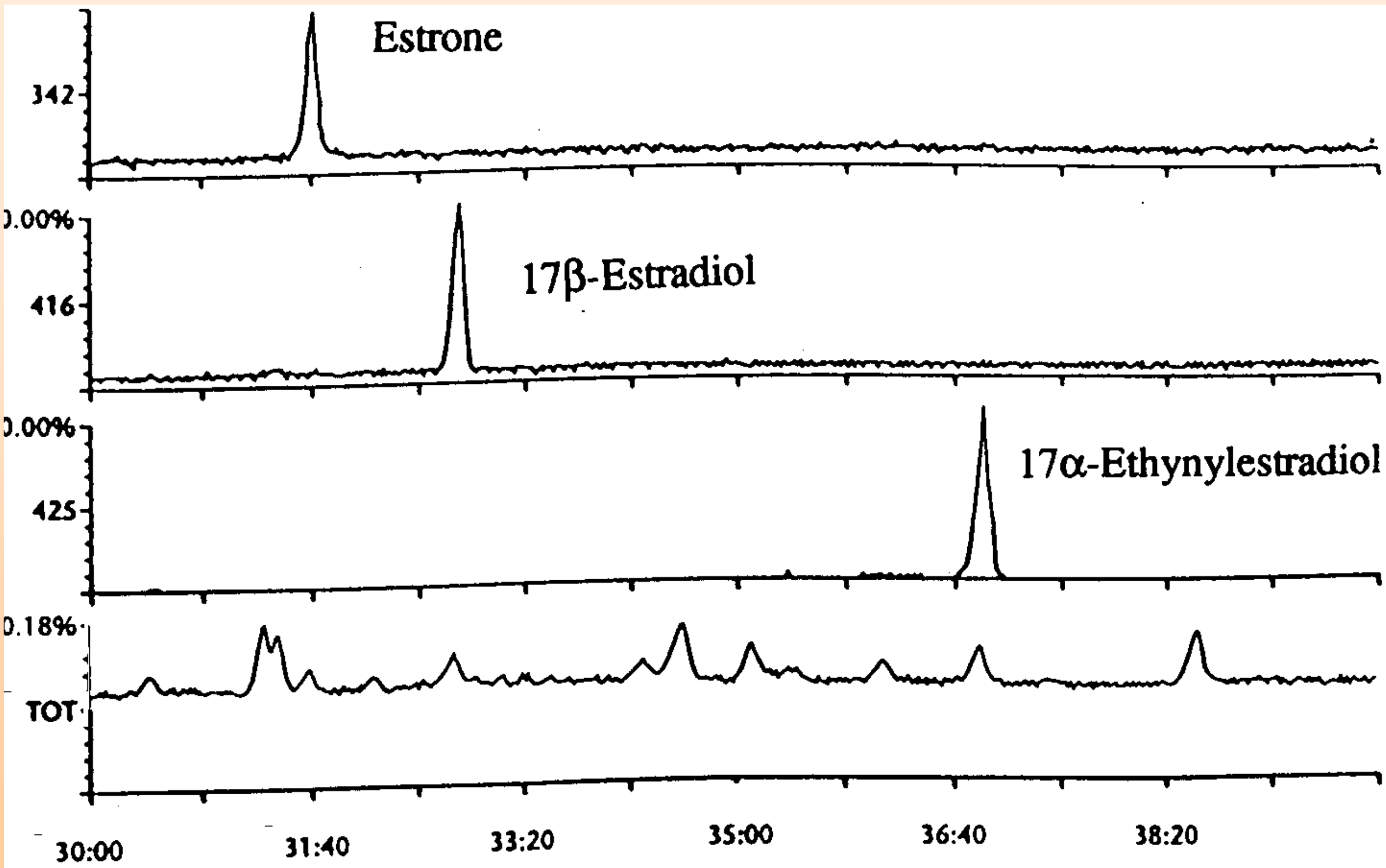


Environmental Estrogens Responding to Yeast Screen

- **Row A = Bisphenol**
  Row C = Genistein
  Row E = Nonyphenol
  Row F = Octylphenol
  Row H = 17β-Estradiol
  Rows B,D,and G (blank)

- **Deep Red Color indicates Estrogenic Activity**
- **Yellow indicates Background (β-gal)**

Gas Chromatography/Mass Spectroscopy



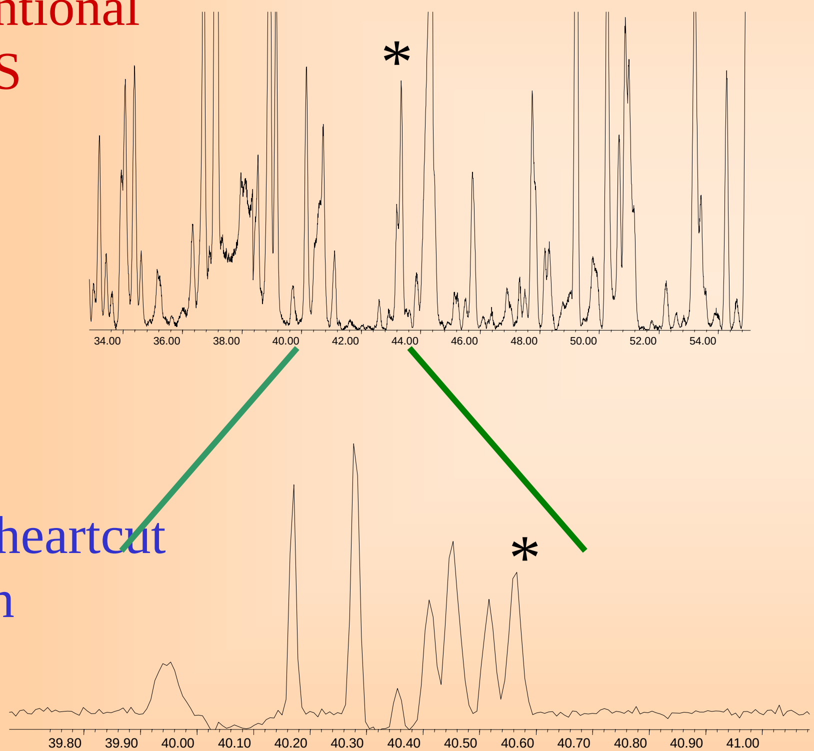
Abundance

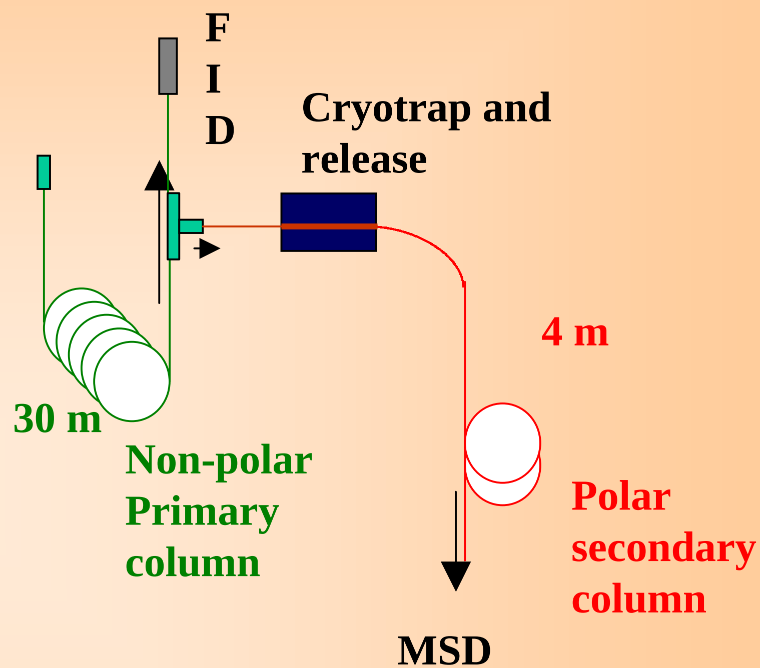
Time (min)

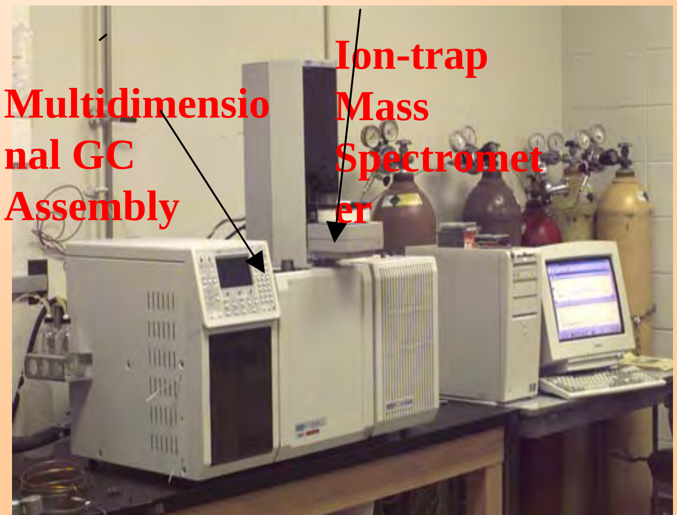
Combustion Pollutant Analysis via Multidimensional Gas Chromatography (courtesy of UDRI)

Conventional GC-MS

MDGC heartcut of region



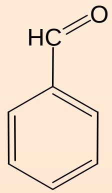




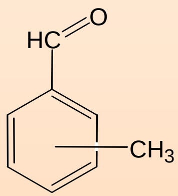
RESULTS

Examples of oxygenates in diesel extracts using MDGC-MS (scanning mode)

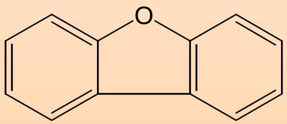
- Benzaldehyde



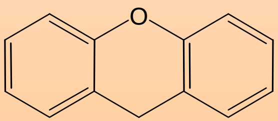
- Methylbenzaldehyde



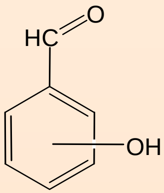
- Dibenzofuran



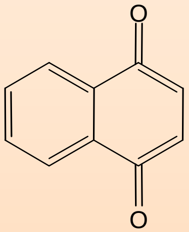
- Xanthene



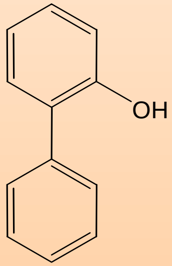
•Hydroxybenzaldehyde



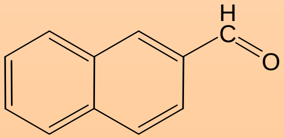
•Naphthalene dione



•Hydroxybiphenyl



•Naphthalene-carboxaldehyde

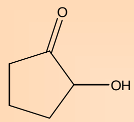


RESULTS

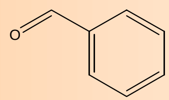
Examples of Oxygenates from Barrel Burn Extract

( ) denotes Match Quality out of 100

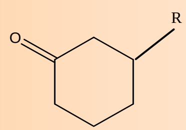
- hydroxy cyclopentanone (7)



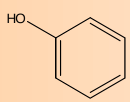
- benzaldehyde (87)



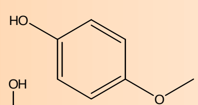
- substituted cyclohexanone (72)



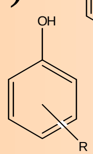
- phenol (80)



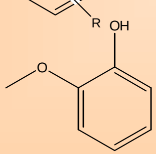
- methyl quinol (90)



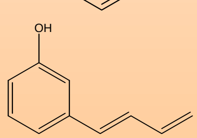
- alkyl phenols (72 - 91)



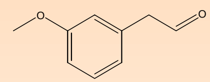
- methoxy methyl phenol (93)



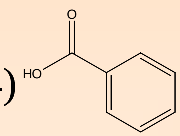
- hydroxy phenyl butadiene (64)



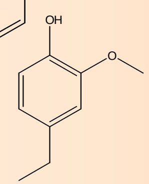
- benzene ethanol 3-methoxy (52)



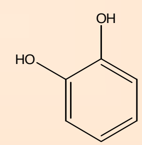
- benzoic acid (64)



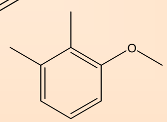
- 4-ethyl-2-methoxy phenol (90)



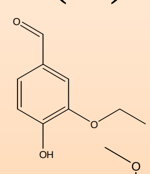
- benzendiol (90)



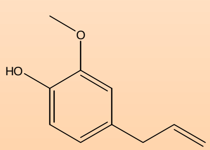
- dimethyl anisole (27)



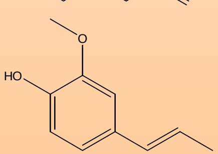
- eugenol (96)



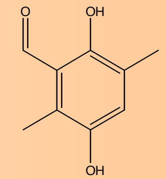
- ethyl vanillin (5)



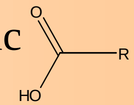
- methoxy propenyl phenol (90)



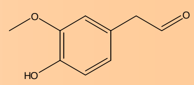
- dihydroxy dimethyl benzaldehyde (5)



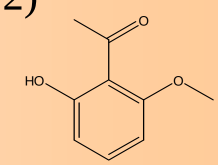
- long chain carboxylic acids



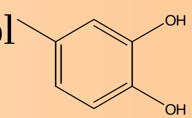
- Hydroxy methoxy phenyl ethanone (72)



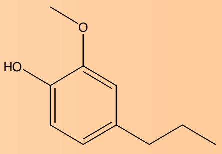
- hydroxy methoxy phenyl propanone



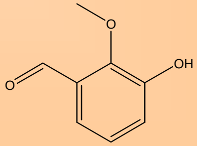
- methyl benzenediol (78)



- methoxy propyl phenol (64)



- vanillin (78)

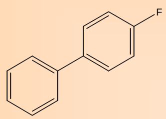


RESULTS

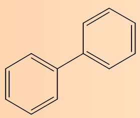
Examples of PAH from Barrel Burn Extract

(   ) denotes Match Quality out of 100

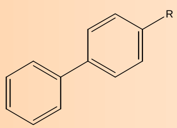
•fluoro-biphenyl (72)



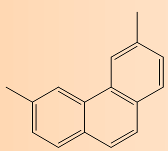
•biphenyl (83)



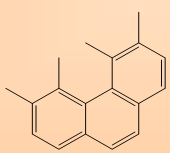
•me-biphenyl (80)



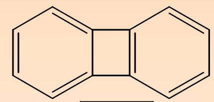
•dimethyl phenanthrene (64)



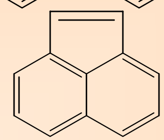
•tetramethyl phenanthrene (38)



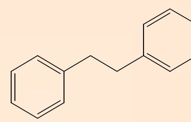
•biphenylene (72)



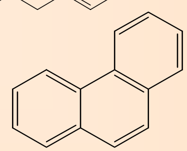
•acenaphthalene (80)



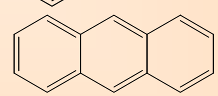
•bibenzyl (74)



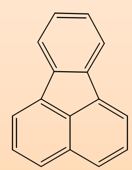
•phenanthrene or



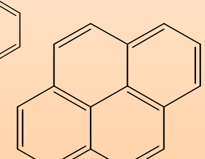
anthracene (72)



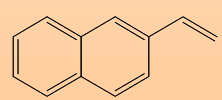
•fluoranthene or



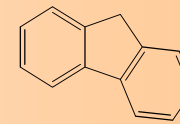
pyrene (72)



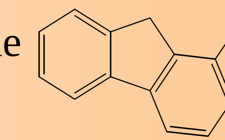
•vinyl naphthalene (48)



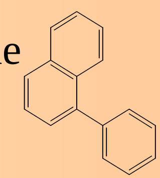
•fluorene (90)



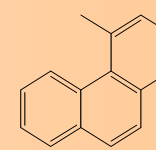
•methyl fluorene (74)



•phenyl naphthalene (69)



•methyl phenanthrene (43)

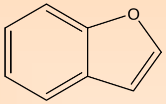


RESULTS

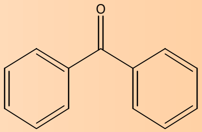
Examples of Oxy-PAH from Barrel Burn Extract

( ) denotes Match Quality out of 100

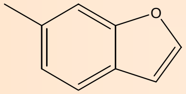
- benzofuran (83)



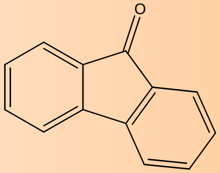
- benzophenone (87)



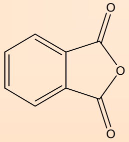
- methyl benzofuran (72)



- fluoren-one (72)



- phthalic anhydride (86)



ENDOCRINE DISRUPTORS FROM COMBUSTION AND VEHICULAR EMISSIONS: IDENTIFICATION AND SOURCE NOMINATION

Brian Gullett[1*], Clyde Owens[1], Jeff Ryan[1], Paul Lemieux[1],
Carolyn Acheson[2],
Michael DeVito[3], James Rabinowitz[3],
Sukh Sidhu[4],
Richard Striebich[4], Joy Klosterman[4]

U.S. Environmental Protection Agency
[1]National Risk Management Research Laboratory, Air Pollution Prevention and Control Division (MD-65), Research Triangle Park, NC 27711
[2]National Risk Management Research Laboratory, Cincinnati, OH 45268
[3]National Health and Environmental Effects Research Laboratory, Toxicology Division, Research Triangle Park, NC 27711
[4]University of Dayton Research Institute, 300 College Park, Dayton, OH 45469
*919-541-1534, 919-541-0554 (fax), gullett.brian@epa.gov

Fate of the Endogenous Hormones 17β-Estradiol and Testosterone in Composted Poultry Manure and their Sorption/Mobility in Loam Soil and Sand

Heldur Hakk [a], Patricia Millner [b], and Gerald Larsen [a]

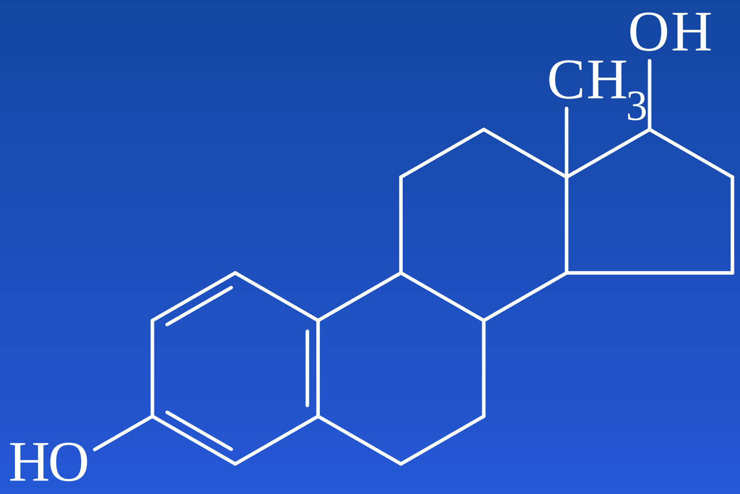
Colleen Pfaff [a], Barb Magelky [a], and Frank Casey [c]

[a] USDA-ARS Biosciences Research Lab, Fargo, ND

[b] USDA-ARS Soil Microbial Systems Lab, Beltsville, MD

[c] North Dakota State University, Dept. Soil Science, Fargo, ND

Estradiol and Testosterone



Estradiol

- Native hormone for estrogen receptor
- Most potent steroidal estrogen hormone
- 10^4-10^6 times as potent as active APP's
- Excreted in mammals/birds in urine/feces
- Derivatives administered as growth promoters
- Carcinogenic (breast cancer)

Toxicology of Estradiol

- 5-500 ng/L E in water increased alfalfa growth
- 5000-500,000 ng/L E decreased alfalfa growth
- 300 ppb E in poultry litter fed to heifers caused premature udder development
- 1-10 ng/L E increases vitellogenin, inhibits testicular growth in trout, feminization
- 50-300 ppb E resulted in halted gonads and feeding, and death in brown trout
- 250-5000 ng/L E were 84-100% female salmon; 10-200 ug/L contributed to death

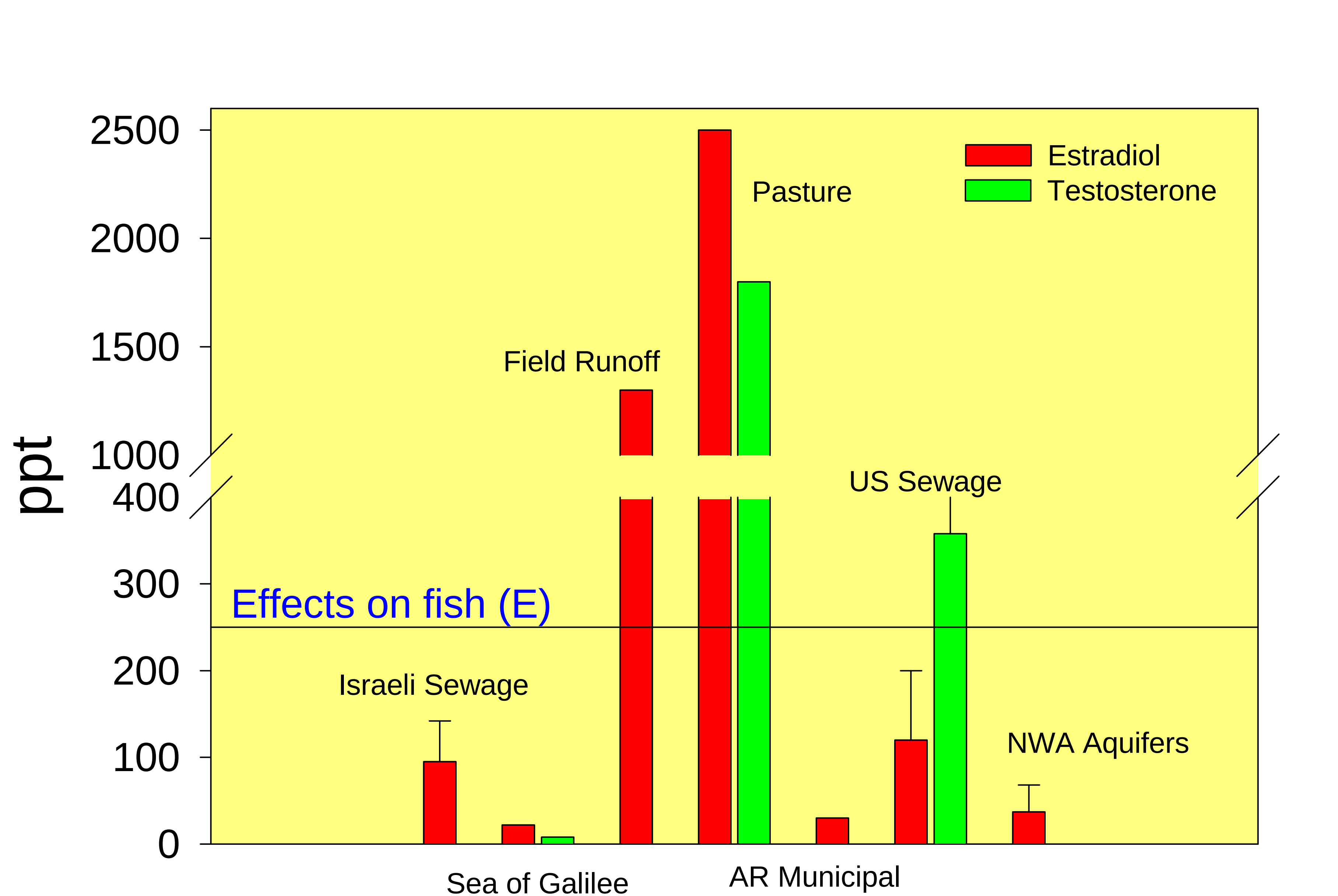
Testosterone

- Native hormone for androgen receptor
- Excreted in mammalian/avian urine and feces
- Responsible for developing and maintaining male sex characteristics
- Reduced to 5α-dihydrotestosterone, which binds receptor with higher affinity
- Serves as a prohormone [T to 17β –E_2 (brain) and T to 5β –diOH T (kidney)]
- Androgenicity of native waters leads to masculinization of mosquitofish, killifish (FL)

Excretion Levels

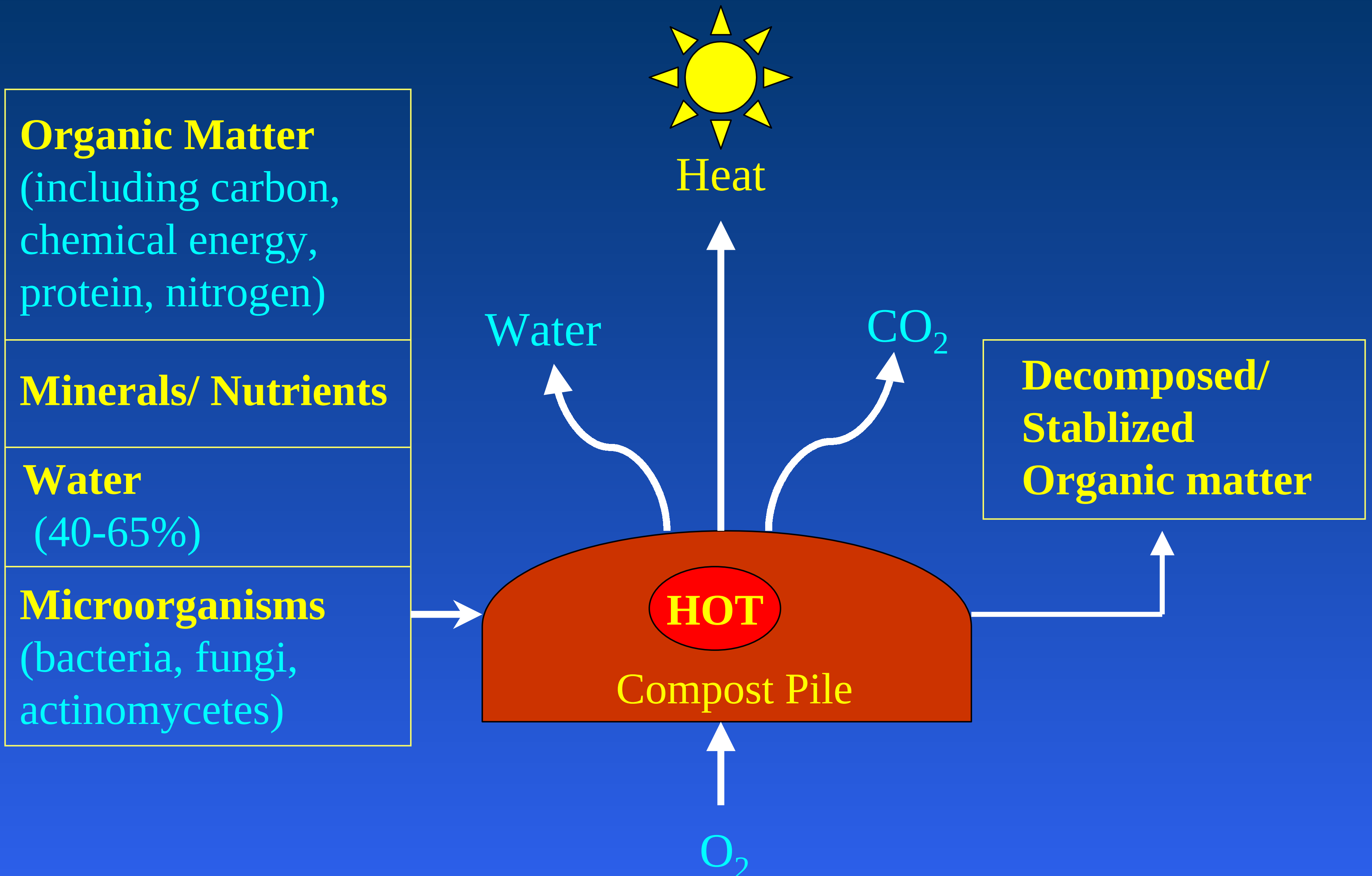
- Adult cattle excrete 30 mg E/day (13 ng/L)
  - Growth hormones increase 5-6 fold in urine
- Chickens excrete 44 ng/g (Ave. dry wt.)
  - 6000 g/yr from chickens on E. shore MD
- Women excrete 25-100 ug/day at ovulation
  - 30 mg/ day E_1, E_2, and E_3 excreted at pregnancy
  - Estrogen replacement therapy prolongs
- Men excrete 2-25 ug/day

Selected Environmental Levels of E and T



The Process of Aerobic Composting

- Microorganisms consume O_2 while feeding on organic matter
- Generates heat, CO_2, and H_2O vapor
- Feedstock can be manure, sludge, leaves, paper, food waste
- Carbon is stabilized
- Finished compost is soil-like, odorless, easy to handle, good storage properties


Organic Matter
(including carbon, chemical energy, protein, nitrogen)
Minerals/ Nutrients
Water
(40-65%)
Microorganisms
(bacteria, fungi, actinomycetes)
Heat
Water
CO_2
HOT
Compost Pile
O_2
Decomposed/ Stablized Organic matter

ARS-BARC Composting Center

- 2 acres
- 8” thick pad
  - Coal ash, cement kiln dust, quicklime, cement
- Surrounded by 8 acres orchardgrass buffer
- Grass and tree buffer zones
- Chesapeake Bay watershed

Composting Center, Beltsville, MD

Composting Methods

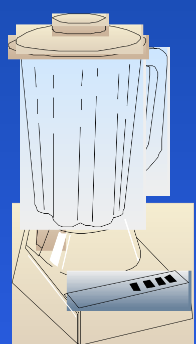
- Chicken layer manure obtained from commercial producer in MD
- Manure adjusted to 30:1 C:N
  - Manure (3.3 parts), old hay (2), straw (2) partially decomposed leaves (4), starter compost (2); [Christiana clay (1)]
- Moisture content maintained at 60%
- Windrows were 160’ x 5’ x 40 inches high
- Windrows turned weekly to aerate
- Three subsamples removed from center weekly (3 wks) then biweekly (16 wks)

Windrow Turner

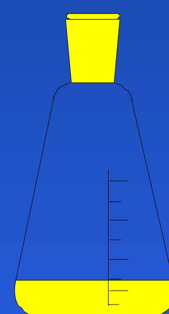
Sample Preparation

Frozen at -20

ELISA

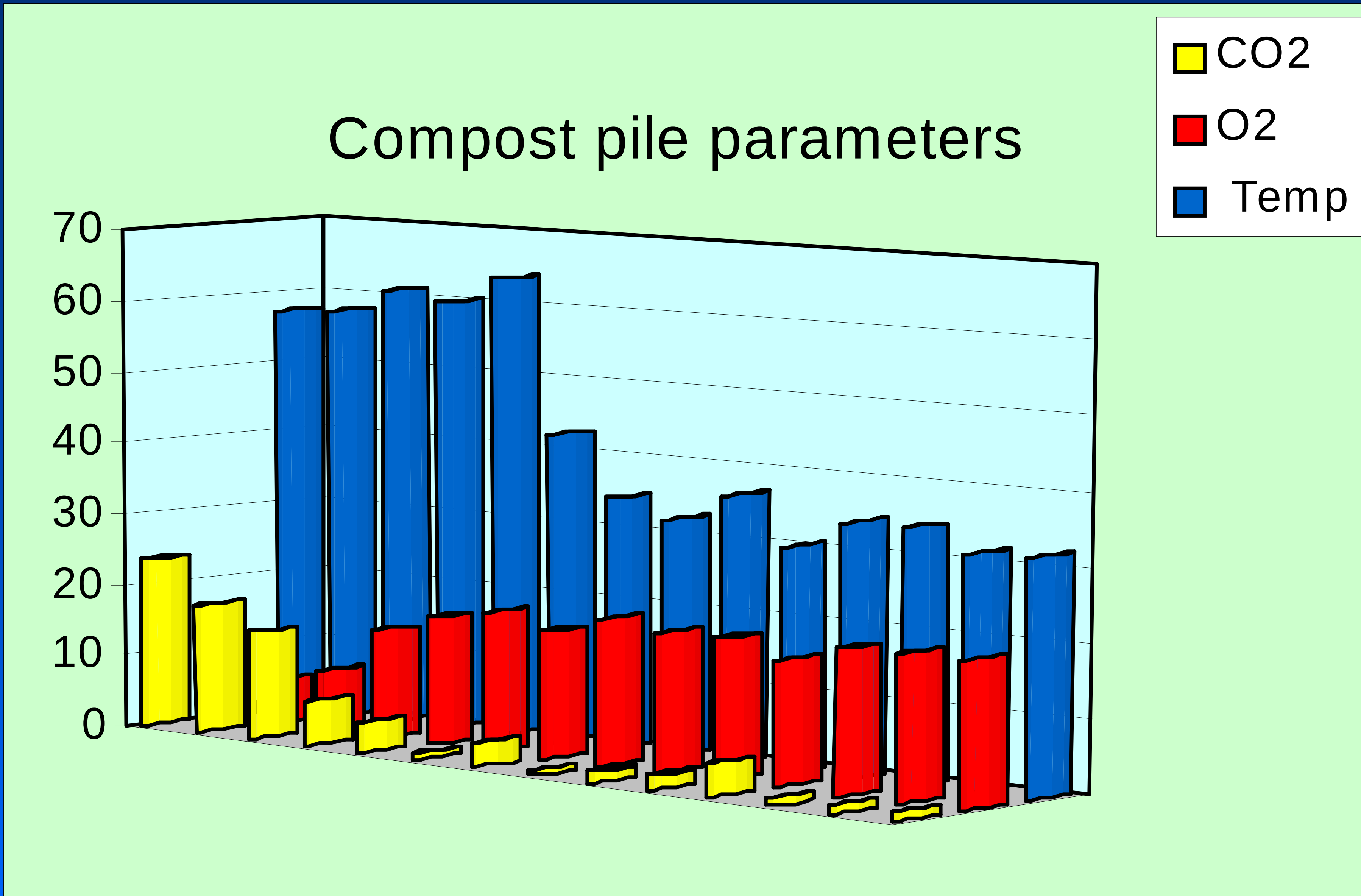


Homogenize with dry ice



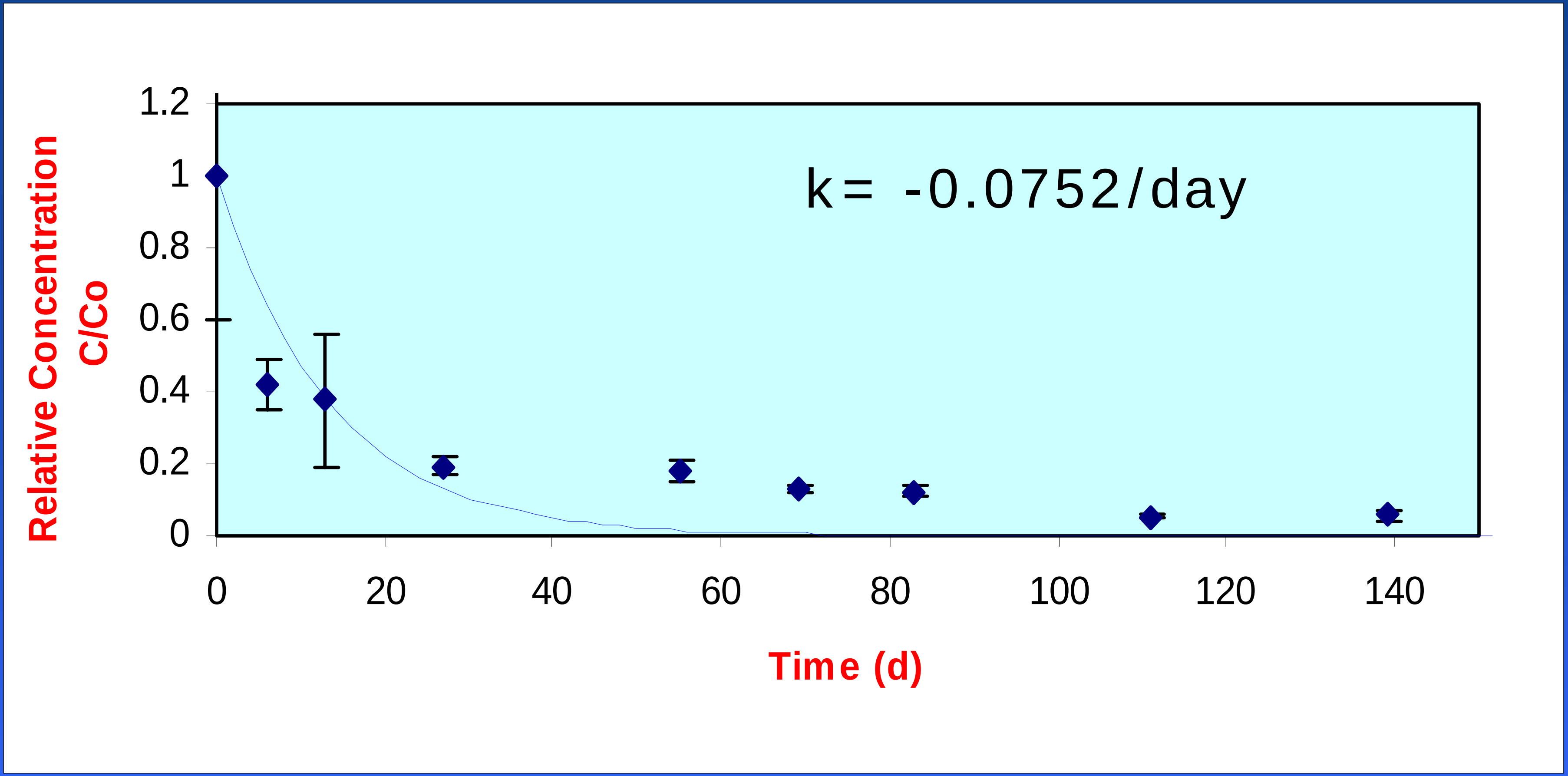
200mg compost/ 50ml water

Centrifuged 15 min @ 2000rpm

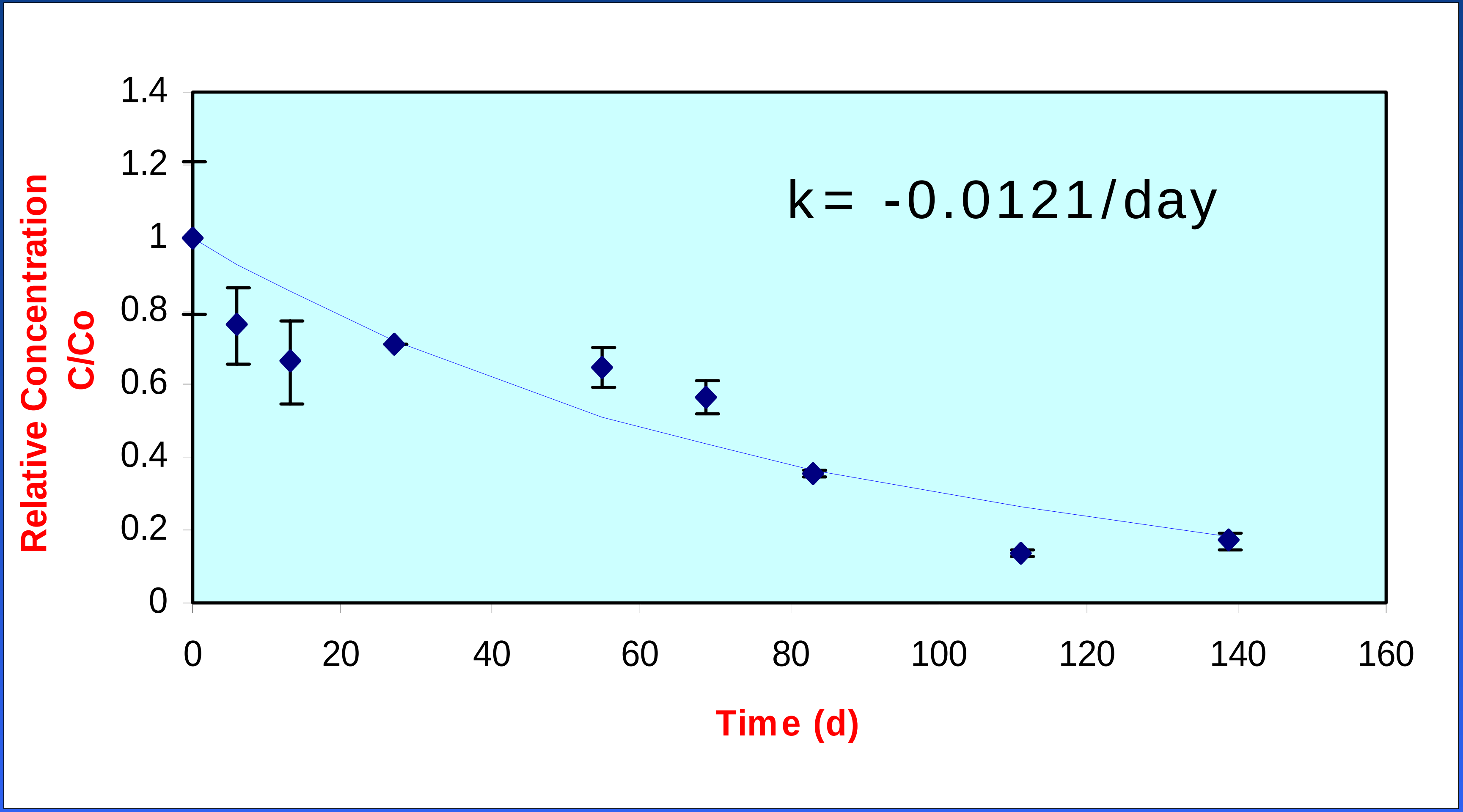


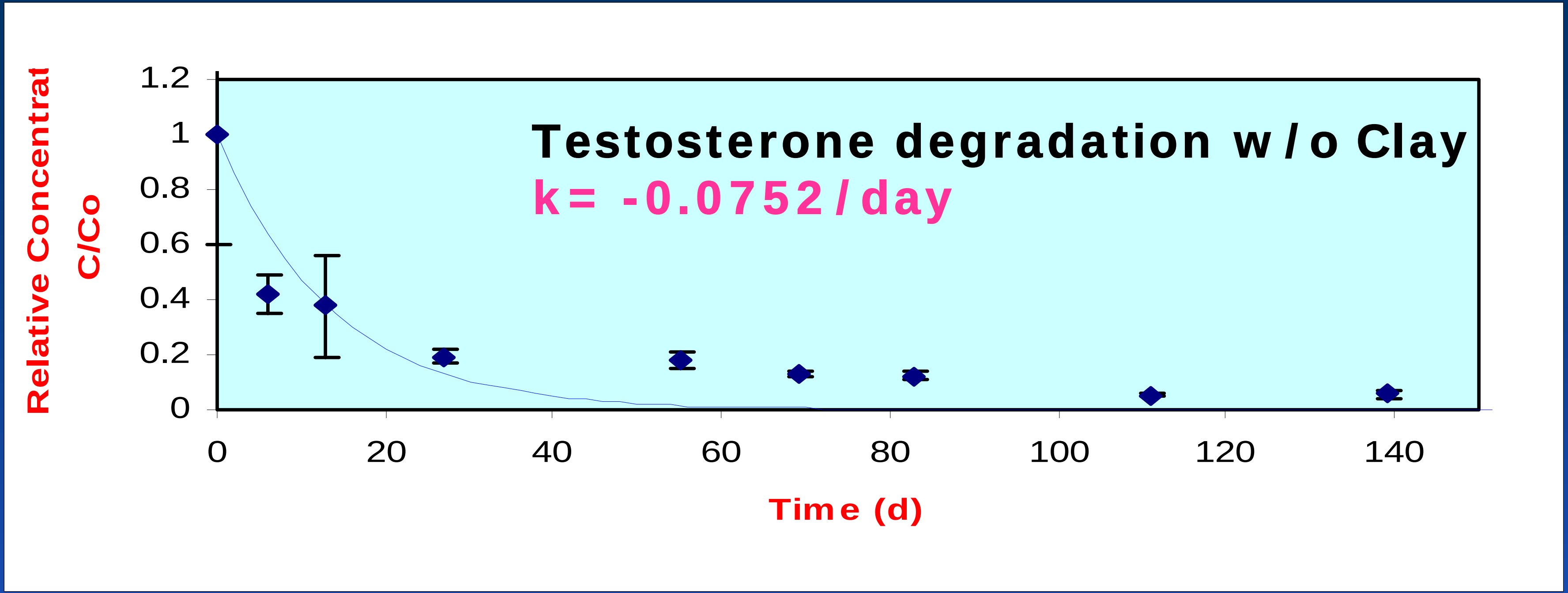
Compost pile parameters
70
60
50
40
30
20
10
0
CO2
O2
Temp

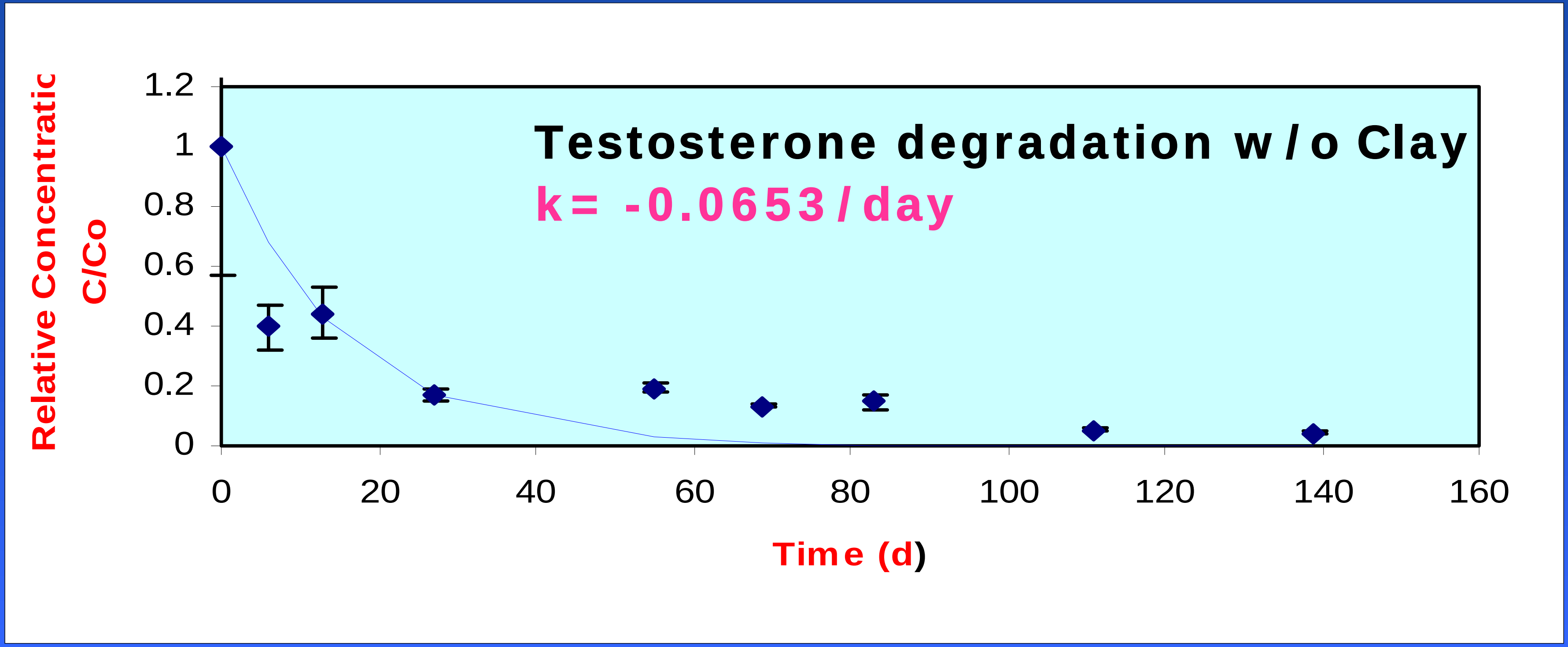
Testosterone Degradation

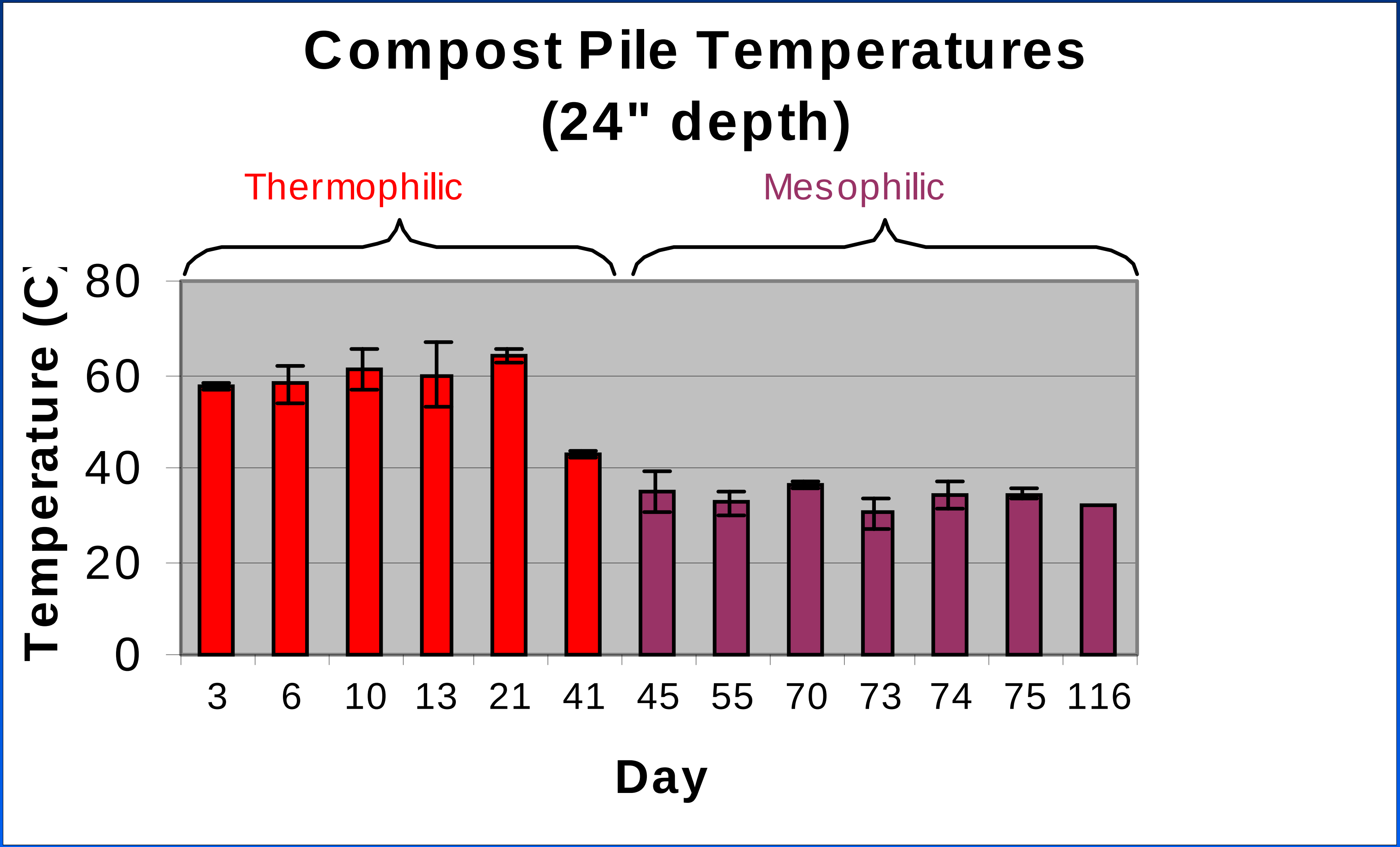


Estradiol degradation




Testosterone degradation w/o Clay
k= -0.0752/day
Relative Concentra
C/Co
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
140
Time (d)


Testosterone degradation w/o Clay
k= -0.0653/day
Relative Concentratic
C/Co
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
140
160
Time (d)



Compost Pile Temperatures
(24" depth)
Thermophilic
Mesophilic
Temperature (C)
80
60
40
20
0
3
6
10
13
21
41
45
55
70
73
74
75
116
Day

Commercial By-Product Derived from Solid Wastes

	Estradiol (ppb)	Testosterone (ppb)
Cattle	19 ± 3.5	n.d.
Swine	152 ± 2.4	21 ± 18
Municipal	31 ± 5.4	29 ± 7.3

Conclusions

- Removable levels of composted chicken manure hormones, estradiol and testosterone, into water were initially 100 & 200 ppb (d.w.), respectively
- Estradiol degradation was observed in composted chicken manure with time
- Testosterone was more rapidly degraded (5-6 fold) than estradiol

Conclusions (Cont'd)

- Two rates of degradation may exist, rapid during thermophilic temperatures, slower during mesophilic
- Neither hormone was degraded completely after 19 weeks, when curing was completed
- Christiana clay amendment did not affect rate of degradation of either hormone

Conclusions (Cont'd)

- Composting may provide an environmentally friendly method of reducing, but not eliminating, the introduction of potent, endogenous hormones into the environment

Batch Studies

1.6g soil/ 8ml of 0.01 $CaCl_2$ at various concentrations; Spike with ^{14}C-hormones

Continuous rotation for 7 days; aliquots taken at 2, 4 and 7 day

Glass column packed with Silt Loam Soil or Sand

8.5cm dia x 15cm

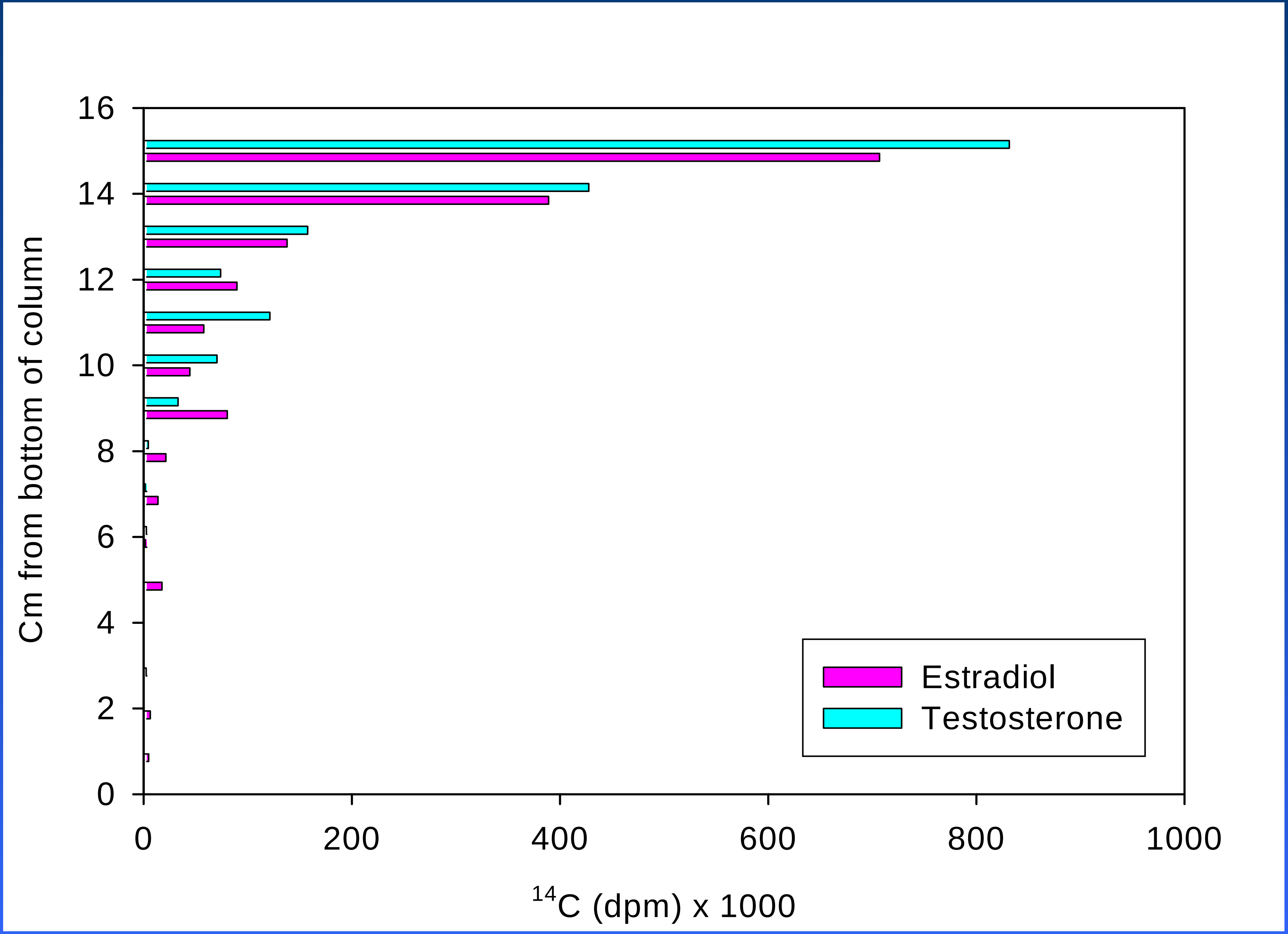
Soil: 1126gms

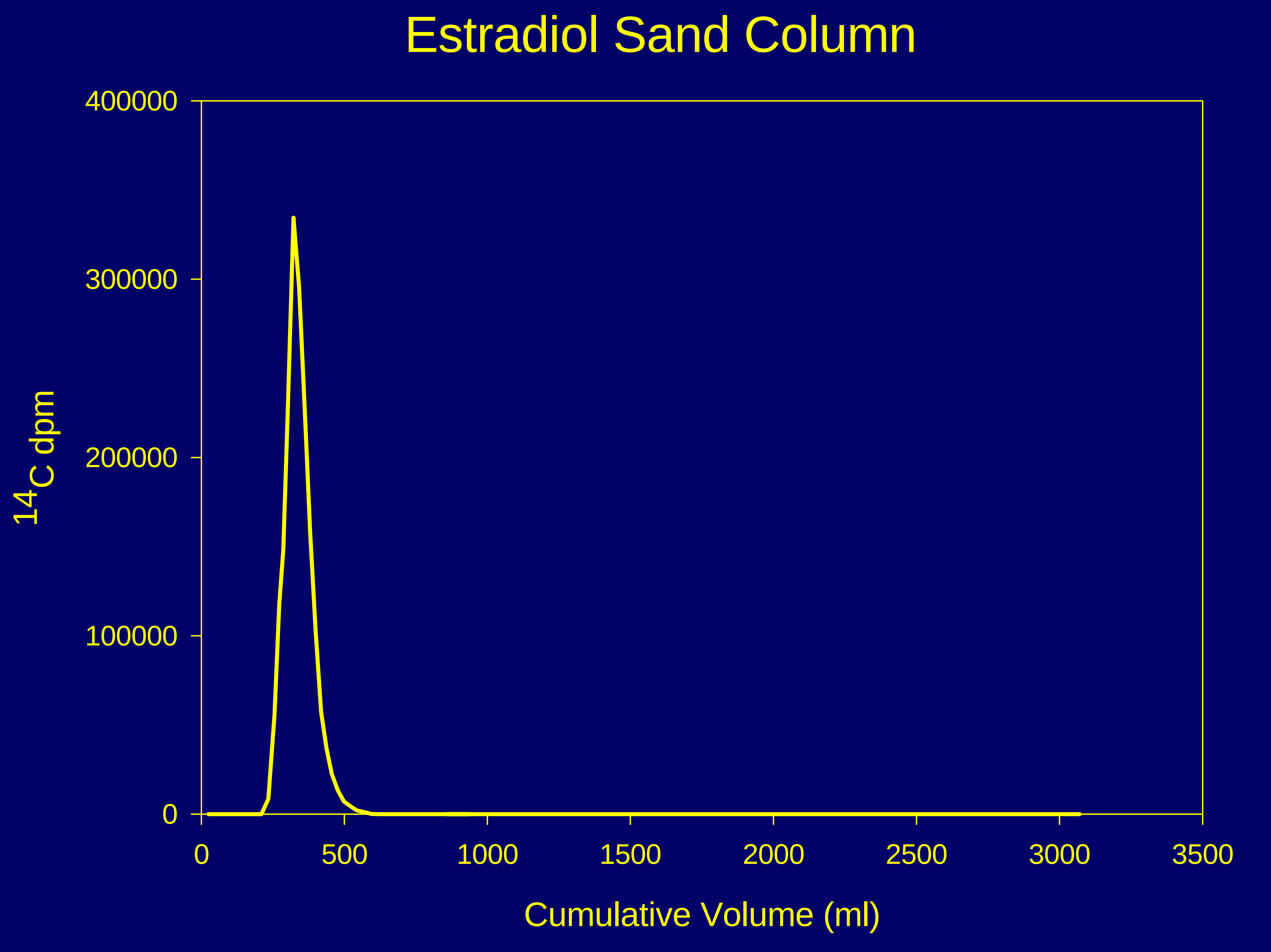
Pore Volume 455ml

Sand: 1427gms

Pore Volume 303ml

[^{14}C]Estradiol and Testosterone at various soil column depths

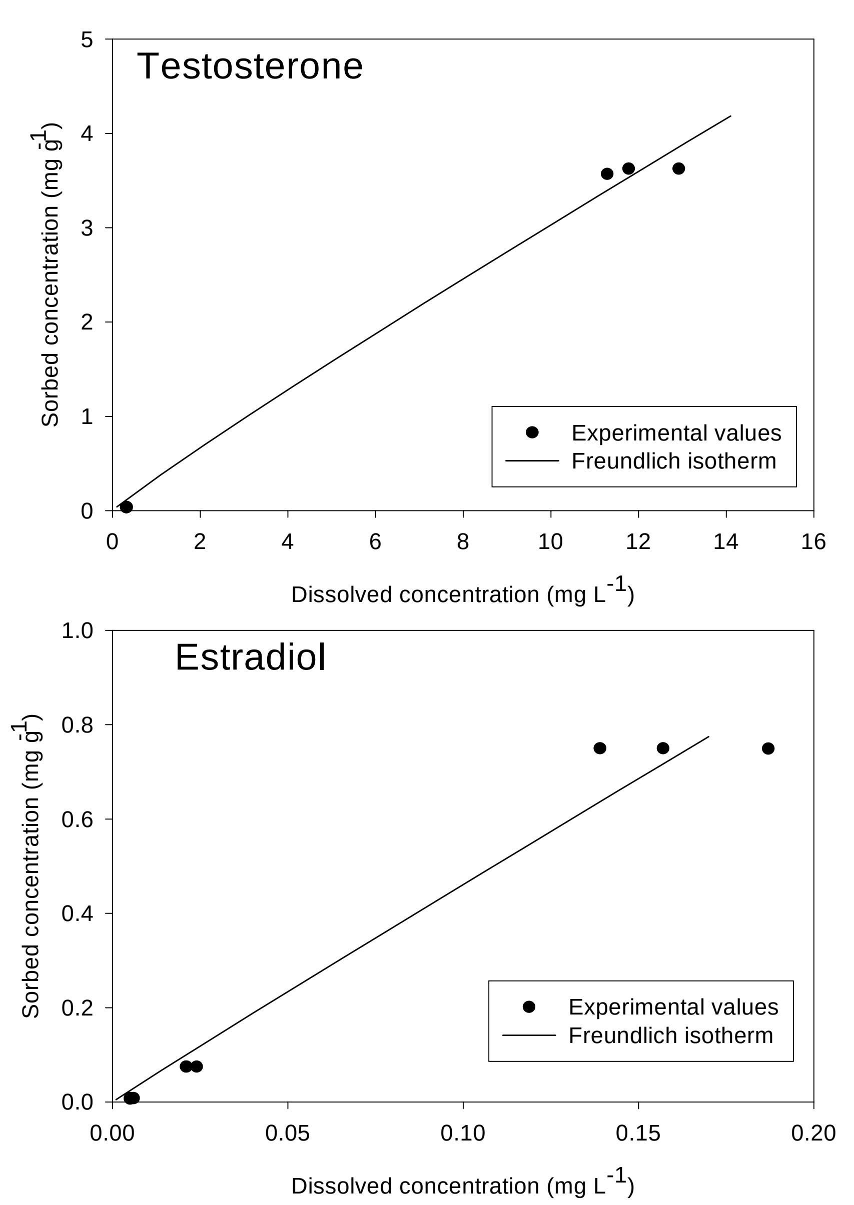



Estradiol Sand Column
400000
300000
200000
100000
0
^{14}C dpm
0
500
1000
1500
2000
2500
3000
3500
Cumulative Volume (ml)

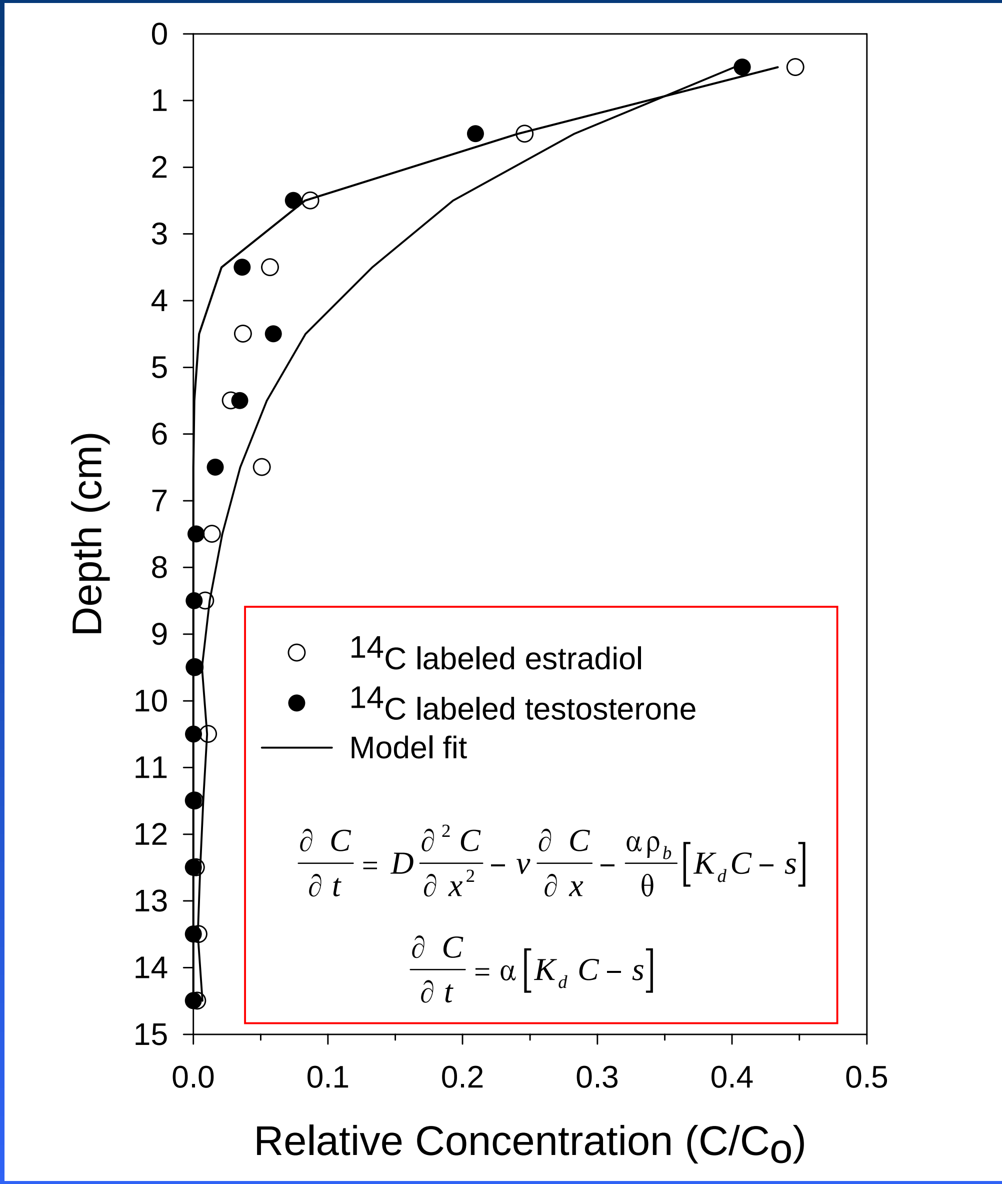
Preliminary Metabolism Data (tlc)

	Soil	Sand
Estradiol	2 metabolites (17-26%)	1 metabolite (85%)
	74-83% bound	
Testosterone	1 metabolite (11-32%)	Parent (88%)
	Parent (5-17%)	
	50% bound	

Batch Studies



Column Studies



K_d Values (cm^3/g)

	Estradiol	Testosterone
Column[a]	1661	182
Batch[b]	4388	348

a Column flow approximately one dimensional

b Batch soil dispersed by agitation – more soil/chemical interaction

Conclusions

- Estradiol and testosterone are readily transported though sand (i.e. not sorbed to the soil mineral fraction)
- Estradiol and testosterone are rapidly and strongly sorbed to the soil (i.e. sorbed to the soil organic fraction)
- Preliminary data indicate estradiol and testosterone may be metabolized in soil
- K_d values derived from batch studies were comparable with soil column results

Future Work

- Express degradation data on a combustible carbon basis to normalize
- Conduct degradation studies with laboratory composters using radiolabeled hormones to determine chemical fate of hormones
- Prolong thermophilic heating phase in order to increase overall degradation
- Conduct composting studies with swine and cattle manure

Potential of Concentrated Animal Feed Operations (CAFOs) To Contribute Estrogens to the Environment

Principal Investigator

Stephen R. Hutchins

USEPA NRMRL, Subsurface Protection and Remediation Division

Robert S. Kerr Environmental Research Center, P.O. Box 1198 Ada, OK 74820

Phone: (580) 436-8563 Fax: (580) 436-8703 email: hutchins.steve@epa.gov

Cooperative Agreement Support

James N. Dumont and David M. Janz

Department of Zoology, Oklahoma State University

430 Life Sciences West, Oklahoma State University, Stillwater, OK 74078

Phone: (405) 744-9683 Fax: (405) 744-7824 email: dumontj@okstate.edu

Phone: (405) 744-7593 Fax: (405) 744-7824 email: djanz@okstate.edu

Contract Support

G. Peter Breidenbach and Dennis D. Fine

ManTech Environmental Services, Inc.

Robert S. Kerr Environmental Research Center, P.O. Box 1198, Ada, OK 74820

Phone: (580) 436-8668 Fax: (580) 436-8501 email: breidenbach.peter@epa.gov

Phone: (580) 436-8669 Fax: (580) 436-8501 email: fine.dennis@epa.gov

Concentrated Animal Feed Operations (CAFOs)

- In the United States, an estimated 376,000 animal feed operations confine animals, generating approximately 128 billion pounds of manure each year
- A facility is an animal feed operation (AFO) if animals are stabled/confined, or fed/maintained, for 45 days or more within any 12-month period, and the facility does not produce any crops, vegetation, or forage growth
- Concentrated animal feed operations (CAFOs) are the largest of these and are regulated under the Clean Water Act. CAFOs are generally considered to be operations with more than 1000 animal units (AU)

Manure Available for Land Application, 1997*

Sector	Total Manure (billion pounds)	Percentage Share by > 1000 AU Operations
Cattle	32.9	83%
Dairy	45.5	23%
Swine	16.3	55%
Poultry	33.5	49%

*Office of Wastewater Management (Office of Water/USEPA) Website: (http://cfpub1.epa.gov/npdes/home/cfm)

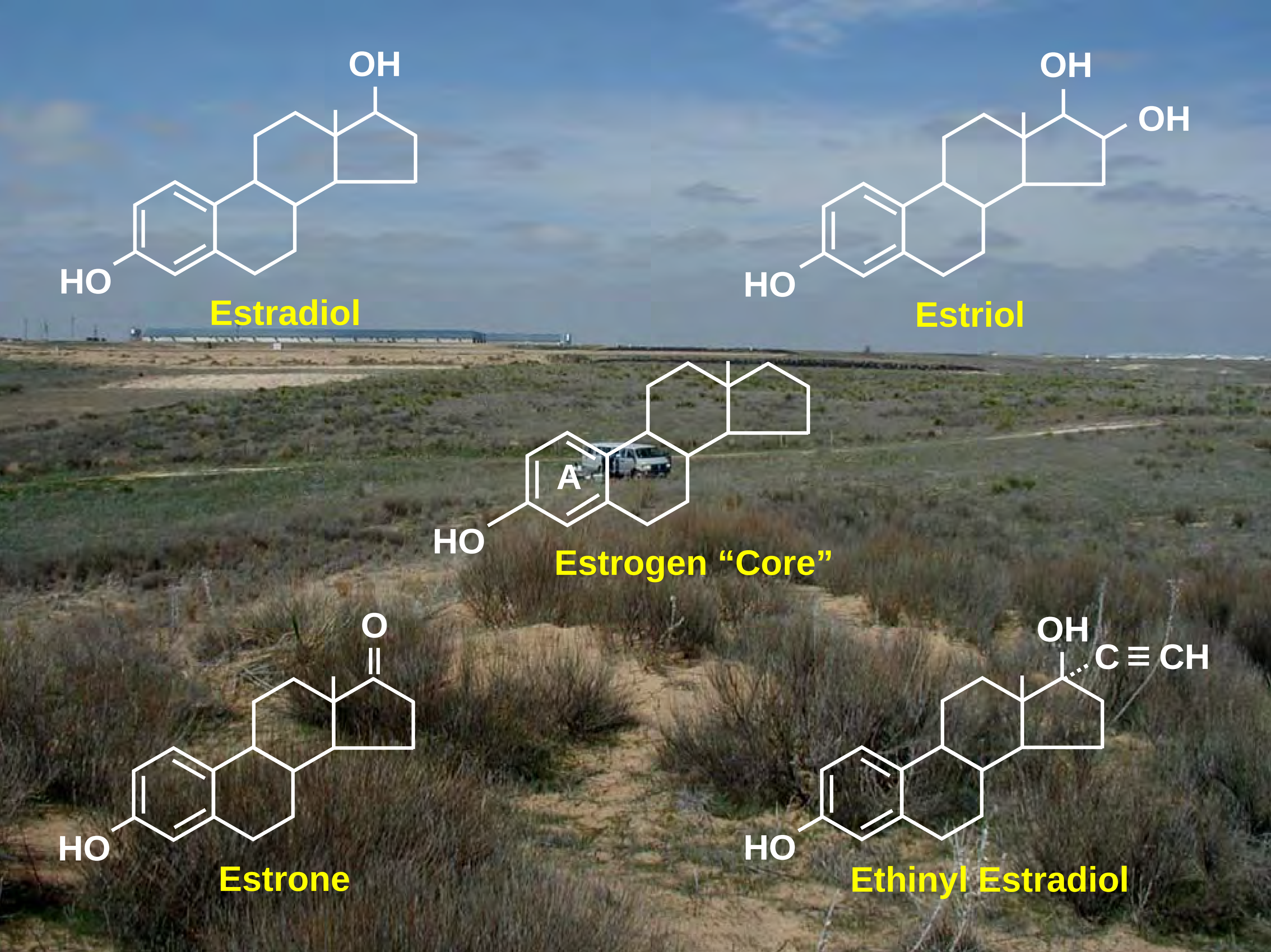
Environmental Estrogens

- **Refer to a wide range of anthropogenic or naturally occurring compounds that elicit estrogenic responses by mimicking endogenous estradiol**

- **Natural Estrogens:**
  - **17β-Estradiol ("Estradiol"), Estriol, Estrone, Equilin, Equilenin, Genistein**

- **Synthetic Estrogens:**
  - **Ethinyl estradiol, Mestranol, Diethylstilbestrol**

- **Other Compounds**
  - ***o, p'* – DDT, Nonylphenol, Bisphenol A**


OH
HO
Estradiol
OH
OH
HO
Estriol
A
HO
Estrogen "Core"
O
HO
Estrone
OH
C≡CH
HO
Ethinyl Estradiol

Comparison of Estrogenic Activity in Terms of EC50 Measured by Yeast Estrogen Screen*

Substance	Relative Ratio of Estrogenic Activity
Natural Estrogens	
Estradiol	1.0
Estrone	0.21
Estriol	0.0013
17β-Estradiol-3-Sulfate	0.000053
Phytoestrogens	
Genistein	0.00011
Other Compounds	
Nonylphenol	0.001
Bisphenol A	0.00027

*Matsui et al, 2000

Comparison of Estrogenic Activity in Terms of Plasma Vitellogenin Induction*

Substance	Minimum Aqueous Concentration Required for Vitellogenin Induction
Natural Estrogens	
Estrone	100 ng/L
Estradiol	10 ng/L
Synthetic Estrogens	
Ethinyl Estradiol	2 ng/L

*Arcand-Hoy et al, 1998

Use and Expected Environmental Impact of Estrogenic Pharmaceuticals Prescribed and Sold in the U.S.*

Pharmaceutical Estrogen Product	Calculated Estrogen Use	Expected Introduction Concentrations to the Aquatic Environment
Human Use		
Oral Contraceptives (ethinyl estradiol, mestranol)	88 kg/yr	2.2 ng/L
Hormone Replacement Therapy (conjugated estrogens)	1700 kg/yr	42 ng/L
Animal Use (Cattle Only)		
Growth-Enhancement (estradiol)	580 kg/yr	14 ng/L

*Arcand-Hoy et al, 1998

CAFO Contributions of Estrogens - Cattle

- Estimated that at least 90% of feedlot cattle slaughtered in 1995 were administered growth-enhancing hormones

- Growth Hormones

  - Estrogens (estradiol, estradiol benzoate)

  - Androgens (trenbolone acetate, testosterone propionate)

  - Progestins (progesterone)

- For cattle, the estradiol concentration in the urine averages 13 ng/L

- Cattle subjected to growth hormones generate urine with estradiol concentrations five- to sixfold greater

CAFO Contributions of Estrogens - Poultry

- No growth hormones added: natural production of estrogens and testosterone

- In 1998, the U.S. poultry industry produced almost eight billion broilers with a total production of almost 12 billion kg litter

- Average estimated hormone concentrations per kg dry weight litter:

  - 14 μg estrogens (estradiol, estrone) in male broilers

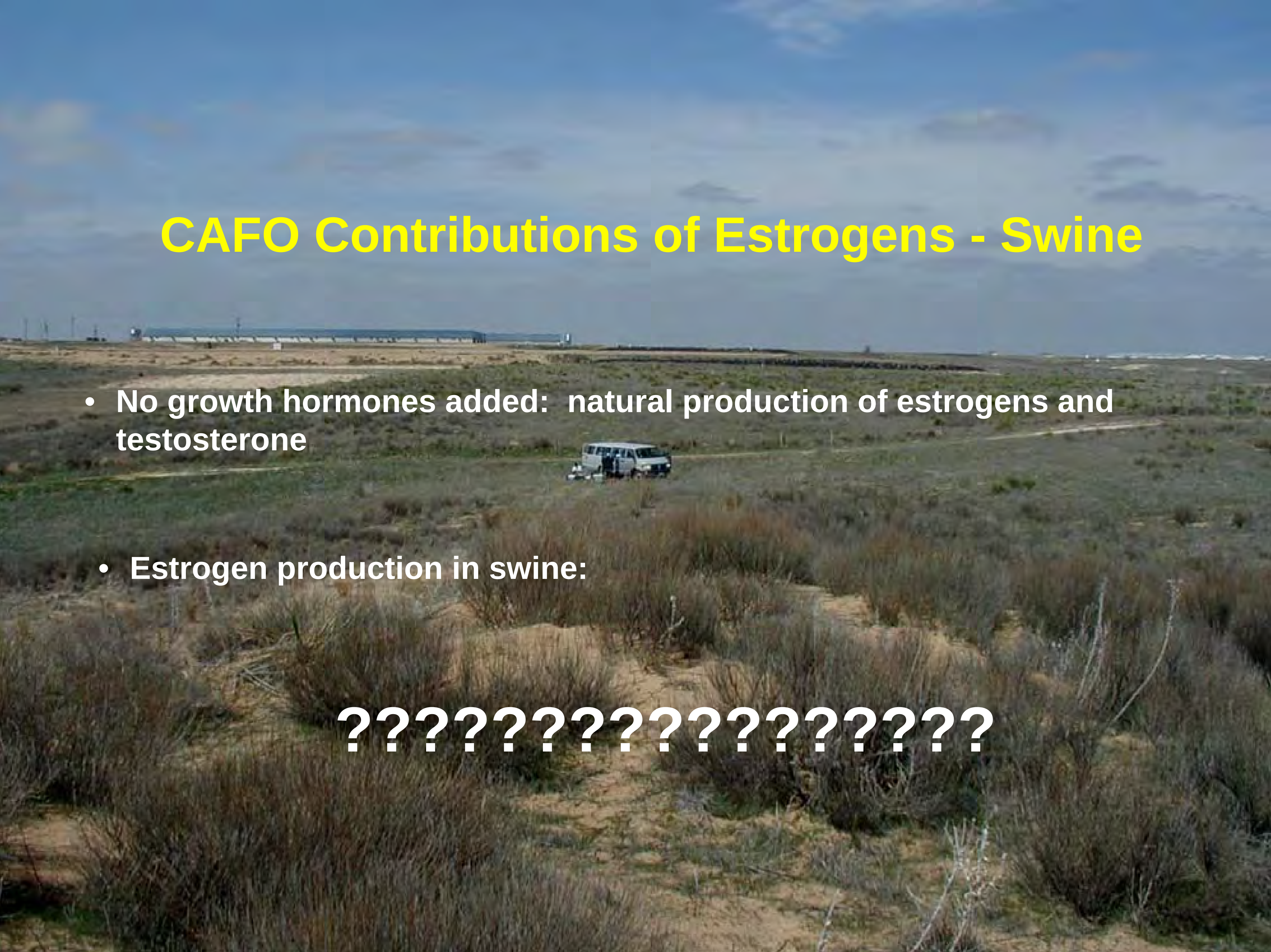
  - 65 μg estrogens (estradiol, estrone) in female broilers

  - 133 μg testosterone in male and female broilers

- Estimated estrogen production: 160,000 – 760,000 kg/year

- Field study shows sizeable edge-of-field losses of estradiol (20-2530 ng/L) and testosterone (10-1830 ng/L) in runoff from litter-amended grasslands (Finlay-Moore et al, 2000)

CAFO Contributions of Estrogens - Swine



- No growth hormones added: natural production of estrogens and testosterone

- Estrogen production in swine:

????????????????

Evaluation of CAFO Lagoon Effluents for EDC Activity using Bioassays

Principal Investigator
James N. Dumont
Department of Zoology
430 Life Sciences West
Oklahoma State University
Stillwater, OK 74078
Phone: (405) 744-9683
Fax: (405) 744-7824
email: dumontj@okstate.edu

Co-Principal Investigator
David M. Janz
Department of Zoology
430 Life Sciences West
Oklahoma State University
Stillwater, OK 74078
Phone: (405) 744-7593
Fax: (405) 744-7824
email: djanz@okstate.edu

OSU CAFO EDC Study - Objective

The objective of this study is to evaluate lagoon samples from swine, beef, and dairy CAFOs for possible EDC activity, using a variety of tests based upon the African Clawed Frog (*Xenopus laevis*)

OSU Swine Lagoon

OSU Beef Lagoon

OSU Dairy Lagoon

OSU CAFO EDC Study – Test Description

FETAX
(Frog Embryo Teratogenesis Assay – *Xenopus*)

Adult Male Frog Exposure -
Vitellogenin Expression
and Estradiol/Testosterone Changes

XTRA
(*Xenopus* Tail Resorption Assay)

OSU CAFO EDC Study – Test Description

FETAX - Frog Embryo Teratogenesis Assay - *Xenopus*

- **Standardized Protocol (ASTM Standard Guide E 1439-91)**
- **96-hr assay that uses early stage *Xenopus* embryos (Stages 8-11, approximately 8-12 hours old)**
- **Measures growth, mortality, and malformation for detection of potential developmentally toxic compounds and mixtures**
- **<u>Not</u> used for detection of EDC activity per se; needed for screening samples for potential toxicity to determine allowable concentrations**

OSU CAFO EDC Study – Test Description

XTRA – *Xenopus* Tail Resorption Assay

- **Tail resorption controlled by thyroxin – measures disruption of thyroid gland**
- **Uses Stage 56/57 (approximately 38/40-day old) *Xenopus* larvae**
- **Four replicate tanks, 10 larvae per tank, water changed twice per week**
- **Larvae photographed every other day; tail length measured by SigmaScan software**
- **Test duration 15-20+ days (until metamorphosis is complete)**

OSU CAFO EDC Study – Test Description

Adult Male Frog Exposure – Vitellogenin Expression and Estradiol/Testosterone Changes

- **Vitellogenin Analysis – Exposure of male oviparous vertebrates to natural and synthetic estrogens can induce synthesis of the phospholipoglycoprotein yolk precursor vitellogenin**
- **Estradiol/Testosterone Analyses – Indicator of alterations in reproductive endocrine homeostasis**

Exposure: Four groups of 5 adult male *Xenopus* exposed for 21 days

- **<u>Untreated Controls</u> – reared in charcoal-filtered water**
- **<u>Positive Plasma Controls</u> – reared in charcoal-filtered water; intraperitoneal injection of 1 mg/kg ethinylestradiol on Days 1, 3, and 6**
- **<u>Positive Aqueous Controls</u> – reared in charcoal-filtered water with 1 mg/L ethinylestradiol**
- **<u>Test Group</u> – reared in CAFO lagoon effluent**

Analyses

- **After exposure, plasma prepared and assayed for vitellogenin using Western immunoblotting and enzyme-linked immunoassay (ELISA), and assayed for estradiol and testosterone using enzyme-linked immunoassay (ELISA)**

OSU CAFO EDC Study – Conclusions

Conclusions are <u>Preliminary</u> – Analyses Pending

- **Although the swine effluent lagoon is quite toxic, none of the lagoons have exhibited significant EDC activity, at least based on these bioassays**

- **EDC activity <u>may be</u> truly insignificant in these CAFO lagoon effluents**

- **However:**

  - **These lagoons may not be truly representative of large-scale commercial operations**

  - **EDC effects on steroid hormone homeostaesis may be more pronounced under long-term exposure**

OSU CAFO EDC Study – Poster Presentation

22nd Annual SETAC Meeting, November 11-15, Baltimore, MD

- ***Lagoon Water from Confined Animal Feed Operations and Amphibian Development***
  Dumont, J.N.*, Oklahoma State University, Stillwater, OK
  Hutchins, S.R., U.S. EPA (NRMRL/SPRD), Ada, OK

- ***Endocrine Modulating Effects of Lagoon Water from Confined Animal Feed Operations on Amphibians***
  Weber, L.P.*, Dumont, J.N., and Janz, D.M., OSU, Stillwater, OK
  Selcer, K.W., Duquesne University, Pittsburgh, PA
  Hutchins, S.R., U.S. EPA (NRMRL/SPRD), Ada, OK

Analysis of Environmental Estrogens in Swine Wastewater, using ELISA, LC/MS/MS, and GC/MS

G. Peter Breidenbach
Research Microbiologist
ManTech Environmental Services, Inc.
Robert S. Kerr Environmental Research Center
P.O. Box 1198
Ada, OK 74820
Phone: (580) 436-8668
Fax: (580) 436-8501
email: breidenbach.peter@epa.gov

Dennis D. Fine
Analytical Chemist
ManTech Environmental Services, Inc.
Robert S. Kerr Environmental Research Center
P.O. Box 1198
Ada, OK 74820
Phone: (580) 436-8669
Fax: (580) 436-8501
email: fine.dennis@epa.gov


RSKERC
ADA, OK


RAW
CENTRIFUGED
CENTRIFUGED
GLASS FIBER FILTER
CENTRIFUGED
GW 0.45 FILTER
1000 mL
PYREX

Environmental Estrogen Analysis – Objective

- The objective of this study is to develop a protocol for screening and analyzing swine lagoon effluent and ground water for estrogens at environmental levels (ng/L)

- Screening will be done using enzyme-linked immunoassay (ELISA) specific for estradiol. Positive samples will then be analyzed for individual estrogens

- Individual estrogens will be analyzed by LC/MS/MS

- LC/MS/MS Interferences - switch to GC/MS

Environmental Estrogen Analysis – ELISA Screen

Initial Procedure

- Solid phase extraction of 250-mL sample using 6-mL ENVI-CARB SPE cartridge
- Sequential washing with water, methanol/acetic acid, and methanol
- Elution with methylene chloride/methanol
- Evaporation to dryness; resuspension with water/methanol to 500 μL
- Direct analysis of 20-μL aliquots by ELISA for estradiol
- Estimate of estradiol concentration using external calibration curve

Environmental Estrogen Analysis – ELISA Screen

Preliminary Findings

- Cross-reactivity: higher concentrations of other estrogens will produce a similar positive response in the estradiol ELISA screen
  - Estriol – Response is 1% that of estradiol
  - Estrone – Response is 1% that of estradiol
  - Ethinyl estradiol – Response is 0.2% that of estradiol
- Swine lagoon effluent appears to exert a positive interference, in that estimated estradiol concentrations can be much higher than those confirmed by direct LC/MS/MS analysis

Environmental Estrogen Analysis – LC/MS/MS Analysis

Initial Procedure (Finnegan TSQ 7000 Mass spectrometer)

- **Analyze same SPE extract prepared for ELISA screening**
- **Micro-liquid chromatography electrospray MS/MS method**
- **2-μL sample loop injection onto 5-μL Zorbax C18SB packed capillary column**
- **Gradient elution with acetonitrile/water**
- **Addition of ammonium hydroxide to column eluent prior to electrospray source to abstract phenoxy proton from the estrogen**
- **Quantitate estrogen concentrations using calibration curves with estrone-d_4 as the internal standard**

Preliminary Results of Swine Lagoon Estrogen Analysis

(all concentrations in ng/L original water sample)

Sample ID	Type	ELISA	LC/MS/MS Analysis			
		estradiol	estriol	estradiol	ethinyl estradiol	estrone
#1	Lagoon	27.2	5?	3?	0	285
#2	Well	0.1	NA	NA	NA	NA
#33	Lagoon	69.0	0?	0?	20	82
#40	Well	1.3	0?	1?	0	0
#41	Lagoon	21.4	0?	0?	0	10
#51	Well	0.3	0?	1?	0	0
#52	Lagoon	26.6	0?	2?	0	5
#63	Lagoon	6.0	2?	3?	2	10
#64	Well	1.3	0?	1?	0	0
#71	Lagoon	31.2	2?	3?	0	85
#72	Lagoon	81.4	2?	0?	0	246
#73	Lagoon	22.8	6?	9?	5	187
#80	Well	0.3	0?	0?	0	0
#81	Lagoon	84.8	0?	0?	0	34
#84	Lagoon	56.0	0?	0?	0	51

* Not Analyzed

Environmental Estrogen Analysis – LC/MS/MS Analysis

Potential Problems

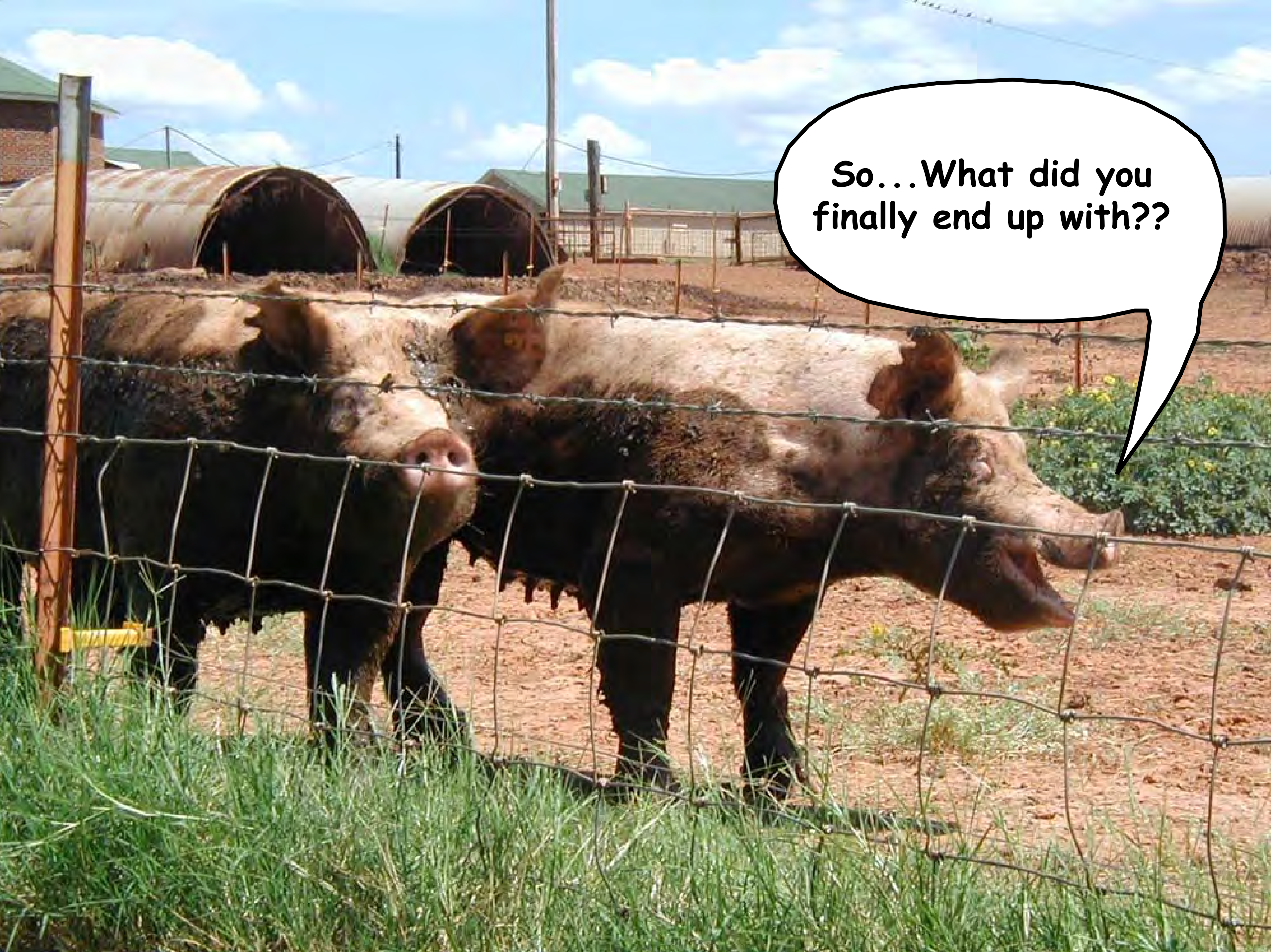
- LC/MS/MS analysis shows numerous organic compounds that elute in early part of chromatograph that may cause positive response with ELISA screen

- Estrogenic response in LC/MS/MS (electrospray) system is suppressed when high organic interferences coelute with compounds of interest (insufficient clean-up)

Possible Solutions

- LC clean-up of complex samples (e.g., swine lagoon effluents) prior to ELISA screen, using gel permeation chromatography and silica gel prep to remove early-eluting interferences

- Evaluate other SPE cartridges and/or alternate analytical techniques


So...What did you finally end up with??

Environmental Estrogen Analysis – SPE Method

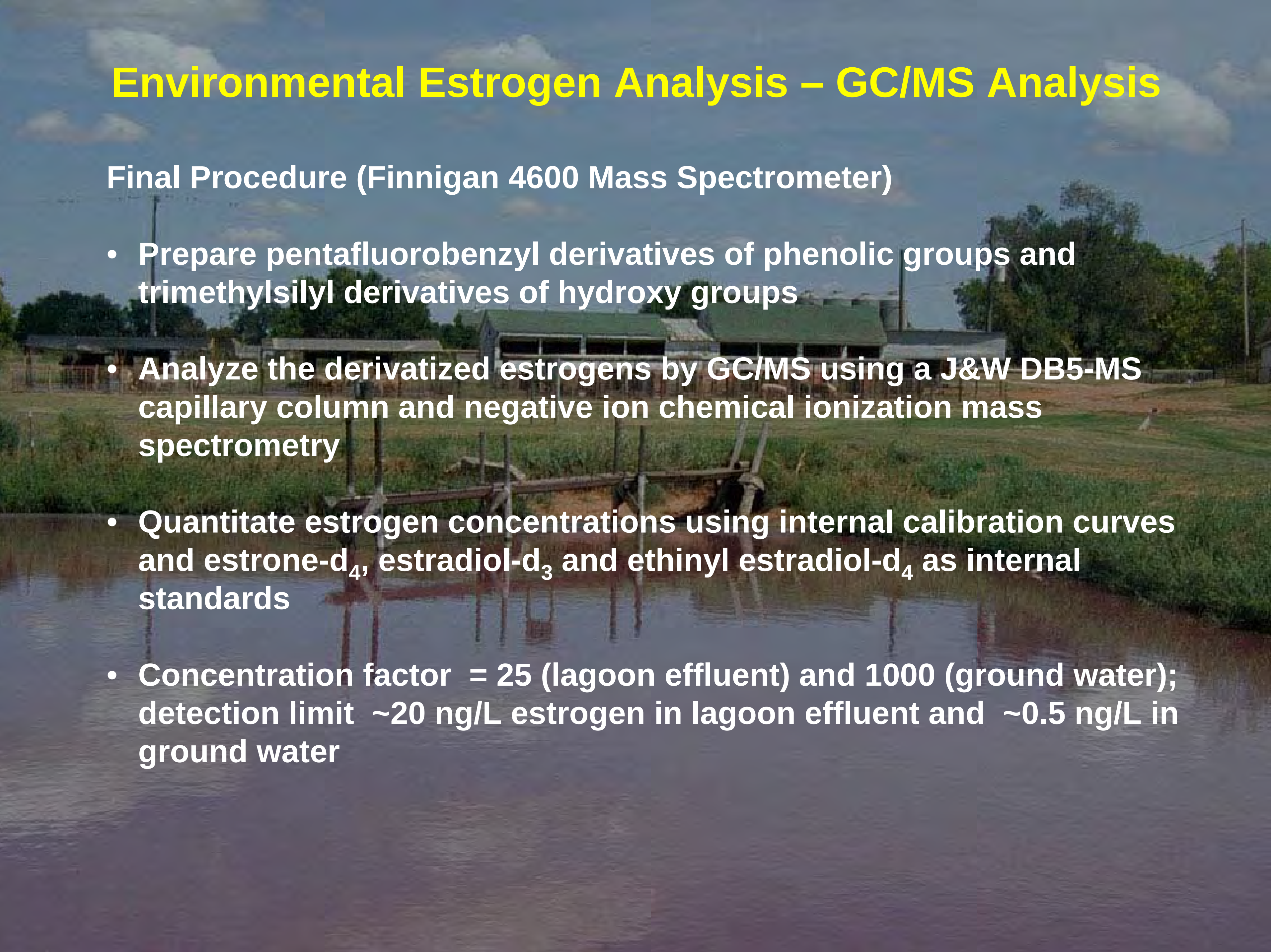
Final Procedure

- **Solid phase extraction of 500-mL water sample or 25-mL swine lagoon effluent sample using OASIS HLB SPE cartridge**
- **Sequential washing with water/methanol, water, and methanol/aqueous ammonium hydroxide**
- **Elution with MTBE/methanol**
- **Evaporation to dryness;**
  - **for ELISA screen, resuspend with methanol to 250 μL and dilute 1:2 with water**
  - **for GC/MS analysis, resuspend with acetone to 1000 μL**

Environmental Estrogen Analysis – ELISA Screen

Final Procedure - used with water samples only

- **Direct analysis of 20-μL aliquots by ELISA for estradiol**
- **Estimate of estradiol concentration using external calibration curve**
- **Concentration factor = 1000; detection limit ~ 0.05 ng/L estradiol in original water sample**



Environmental Estrogen Analysis – GC/MS Analysis

Final Procedure (Finnigan 4600 Mass Spectrometer)

- **Prepare pentafluorobenzyl derivatives of phenolic groups and trimethylsilyl derivatives of hydroxy groups**

- **Analyze the derivatized estrogens by GC/MS using a J&W DB5-MS capillary column and negative ion chemical ionization mass spectrometry**

- **Quantitate estrogen concentrations using internal calibration curves and estrone-d_4, estradiol-d_3 and ethinyl estradiol-d_4 as internal standards**

- **Concentration factor = 25 (lagoon effluent) and 1000 (ground water); detection limit ~20 ng/L estrogen in lagoon effluent and ~0.5 ng/L in ground water**

Recovery of Estrogens Spiked in Distilled Water and in Swine Waste Lagoon Samples

% Recovery	Distilled Water Spiked at 2 ng/L	Distilled Water Spiked at 1 µg/L	Lagoon Effluent Spiked at 1 µg/L (duplicates)	
7-α-Methylestrone (surrogate)	110	85.2	86.2	87.4
Estrone	110	83.2	68.8	58.8
Estradiol	160	84.0	73.6	83.2
Ethinyl estradiol	110	90.8	86.0	88.0
Estriol	210	115	75.4	109

Environmental Estrogen Analysis – GC/MS Analysis

Continuing Work

- **Investigate increasing concentrations of derivatizing reagents to improve quantitation of estriol**
- **Improve recoveries of estrogens by using deactivated glassware and increasing concentrations of derivatizing agents**

Biological Indicators of Exposure of Aquatic Organisms to EDC's

Greg Toth
NERL-EERD

January 29, 2002

Take home messages

- We've developed a specific molecular indicator of exposure to estrogens in the laboratory
- We've measured this indicator in field studies
- We're developing multi-stressor indicator methods using DNA microarray technology

Questions

- What is the extent of exposure of wildlife to EDC's?
  - Mixtures
    - Surface waters and sediment
  - Fish and invertebrates
  - Local and regional scale assessments
- What is the linkage between exposure indicators and effects?
- How effective are risk management practices in reducing EDC's?

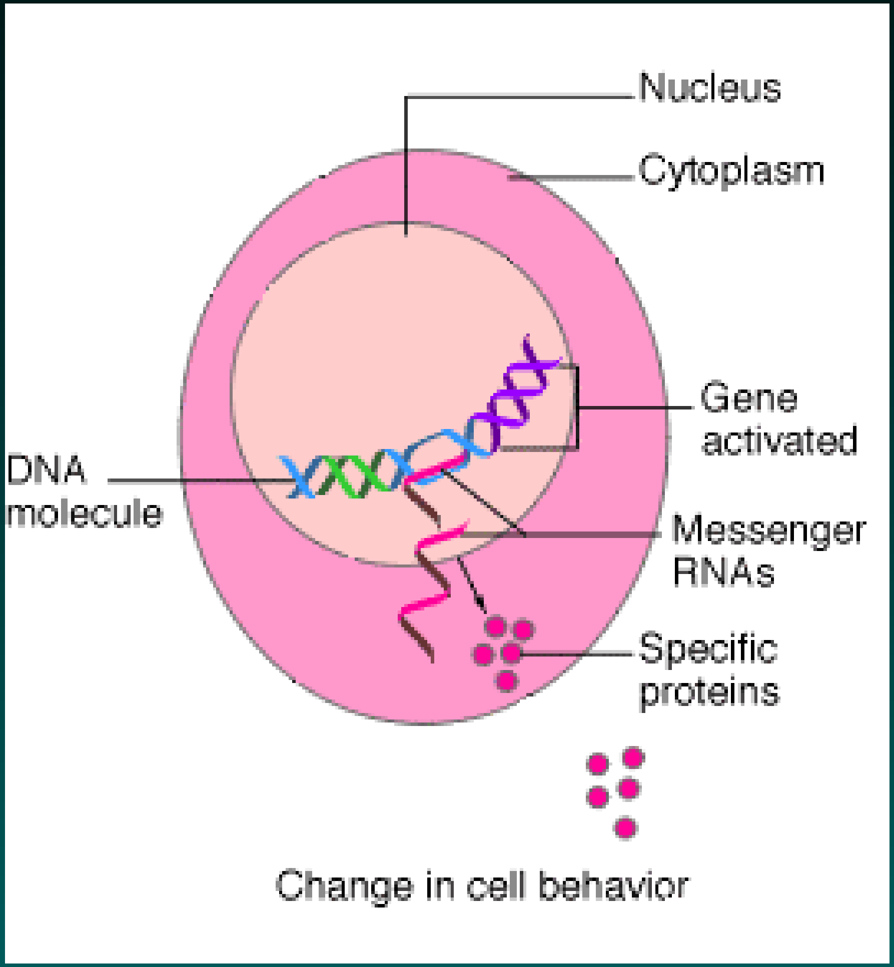
Approaches

- Use of molecular biology to develop new, highly-selective, highly-sensitive indicators
  - Measure changes within cells of organisms exposed to EDC's
    - Measure changes in expression of induced genes
- Collaborate with scientists in the NRMRL, Regions, STAR program and Canada to evaluate performance and assist in the interpretation of indicators in field studies

Resources

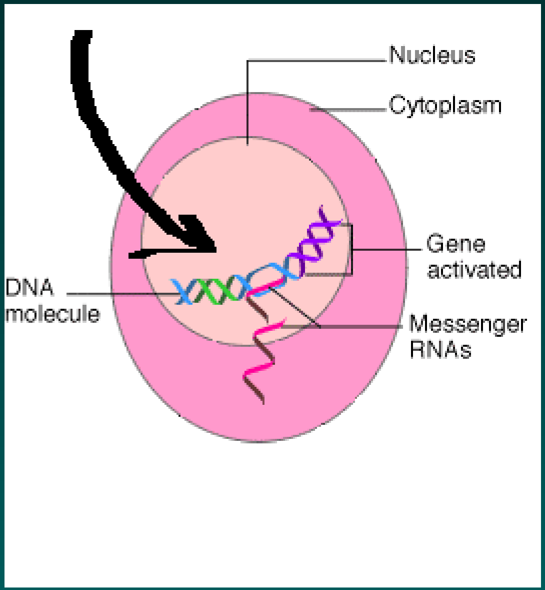
- Core ORD facility for molecular biology
  - 9 Federal scientists work on gene expression
    - 4 Ph.D.'s
      - 2 Molecular biology
      - 1 Aquatic biology
      - 1 Biochemistry
    - 2 M.S.'s
      - Molecular biology
  - Advanced equipment / laboratories / contractors
    - 3 Sequencers
    - DNA microarray scanner

Gene Expression Basics

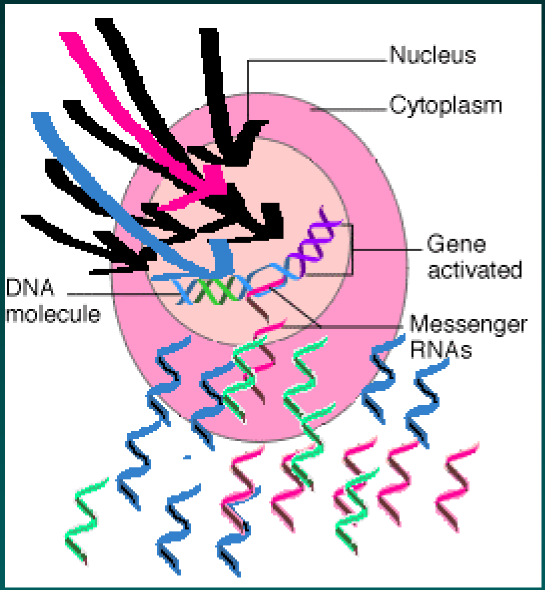


Two Study Themes

- Single stressor / single known gene



- Multiple stressors / multiple genes

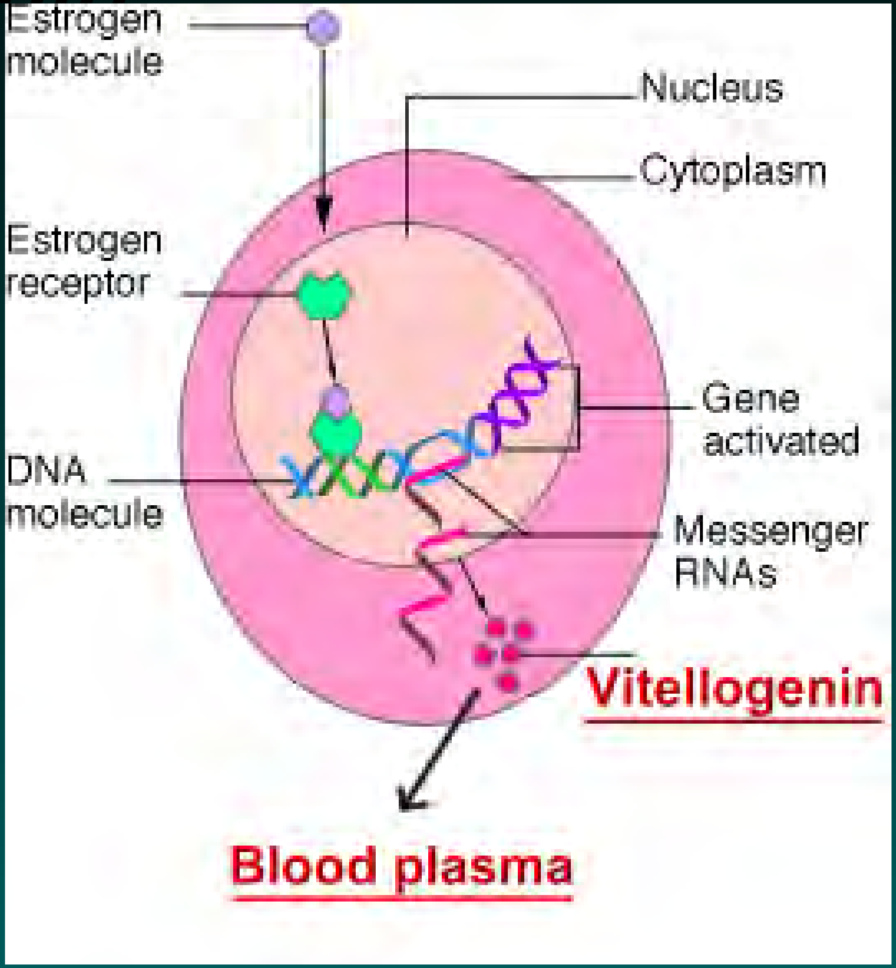


Current Studies

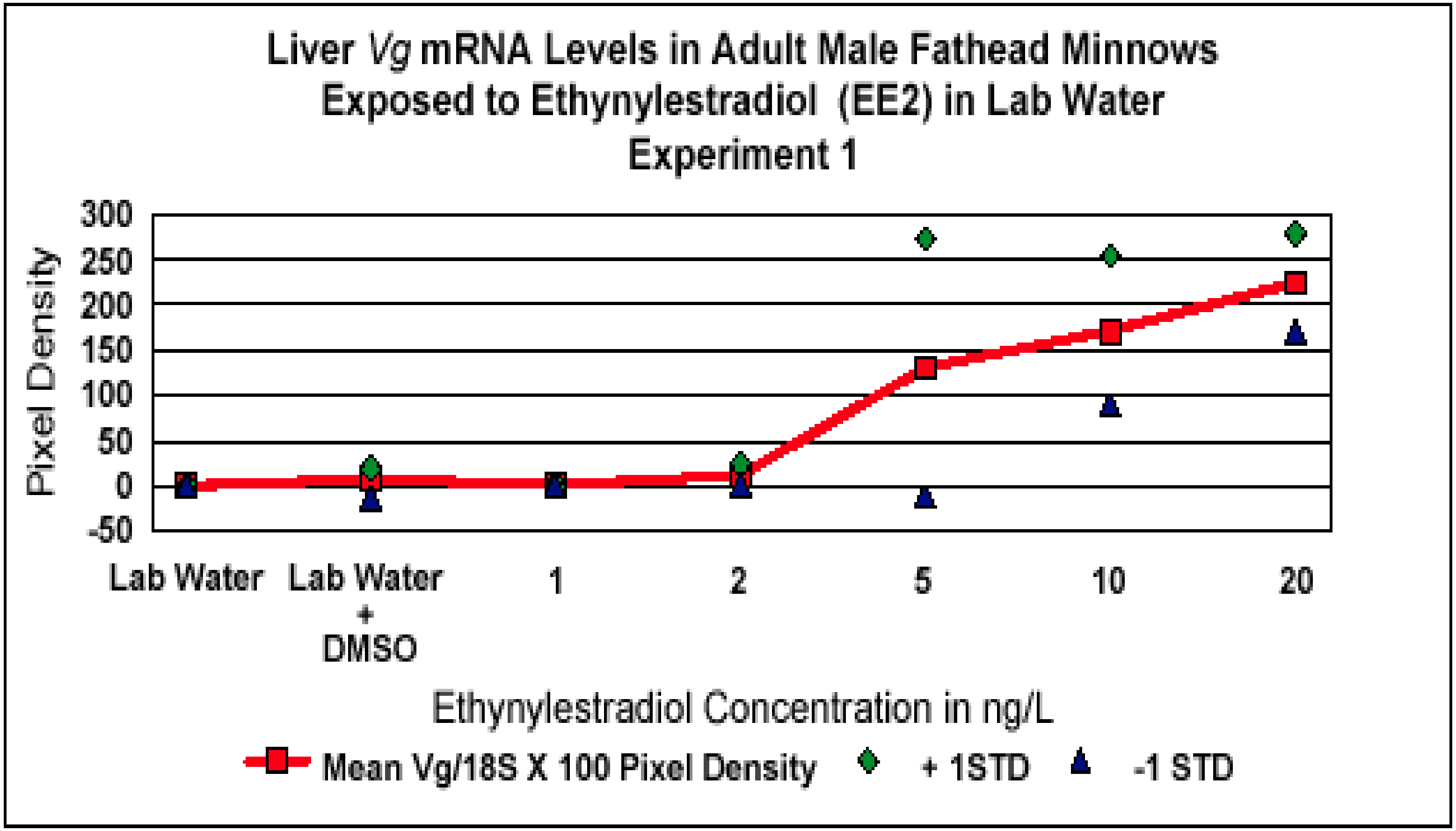
- Single Gene Indicator Studies
  - Laboratory - Adult male and larvae fathead minnows
    - Vitellogenin (*Vg*)

      Estrogens (estradiol, ethynylestradiol, DEHP)

Estrogen-induced Expression of Vitellogenin



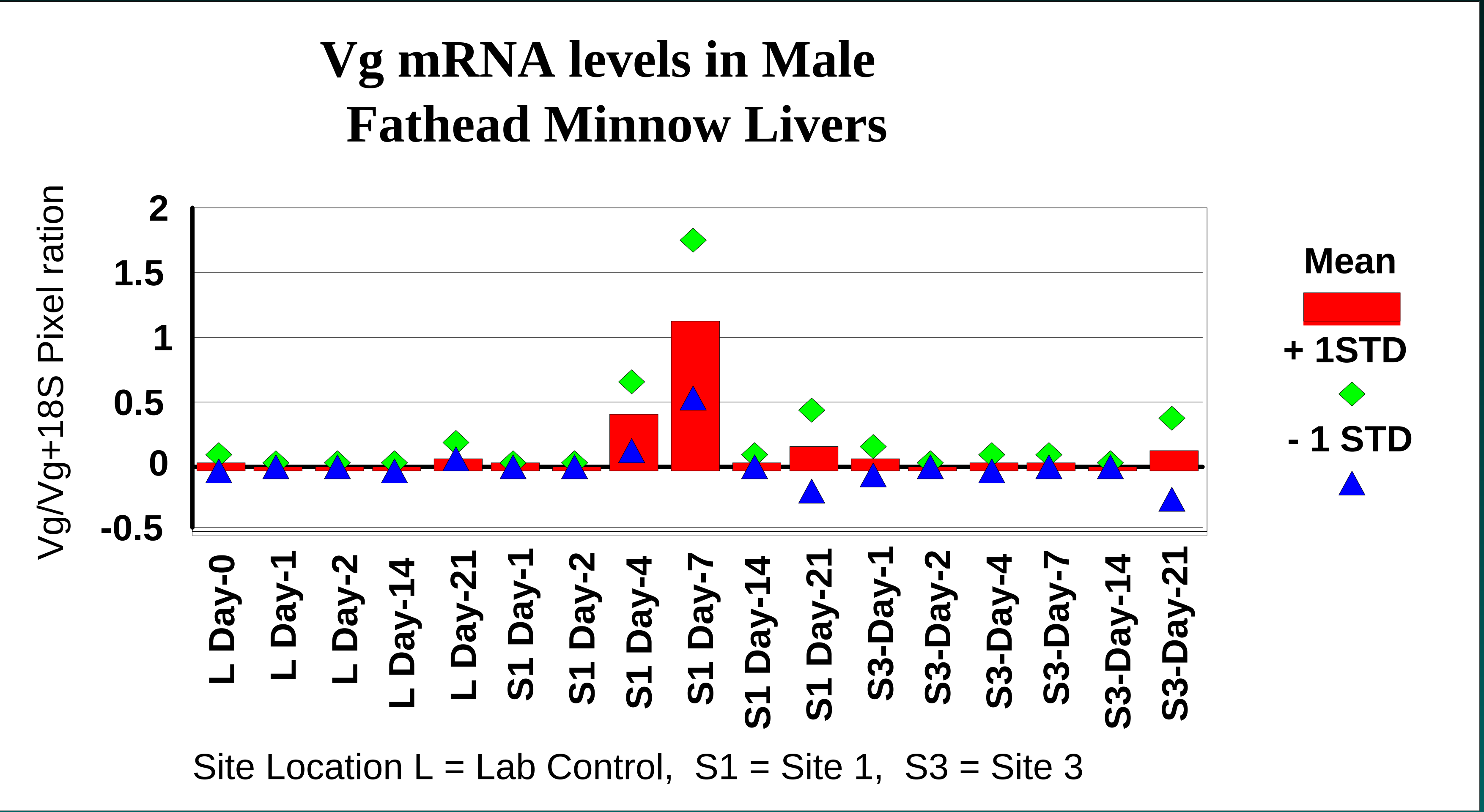
Single Gene Expression
laboratory studies, vitellogenin



Application of Vitellogenin Indicator

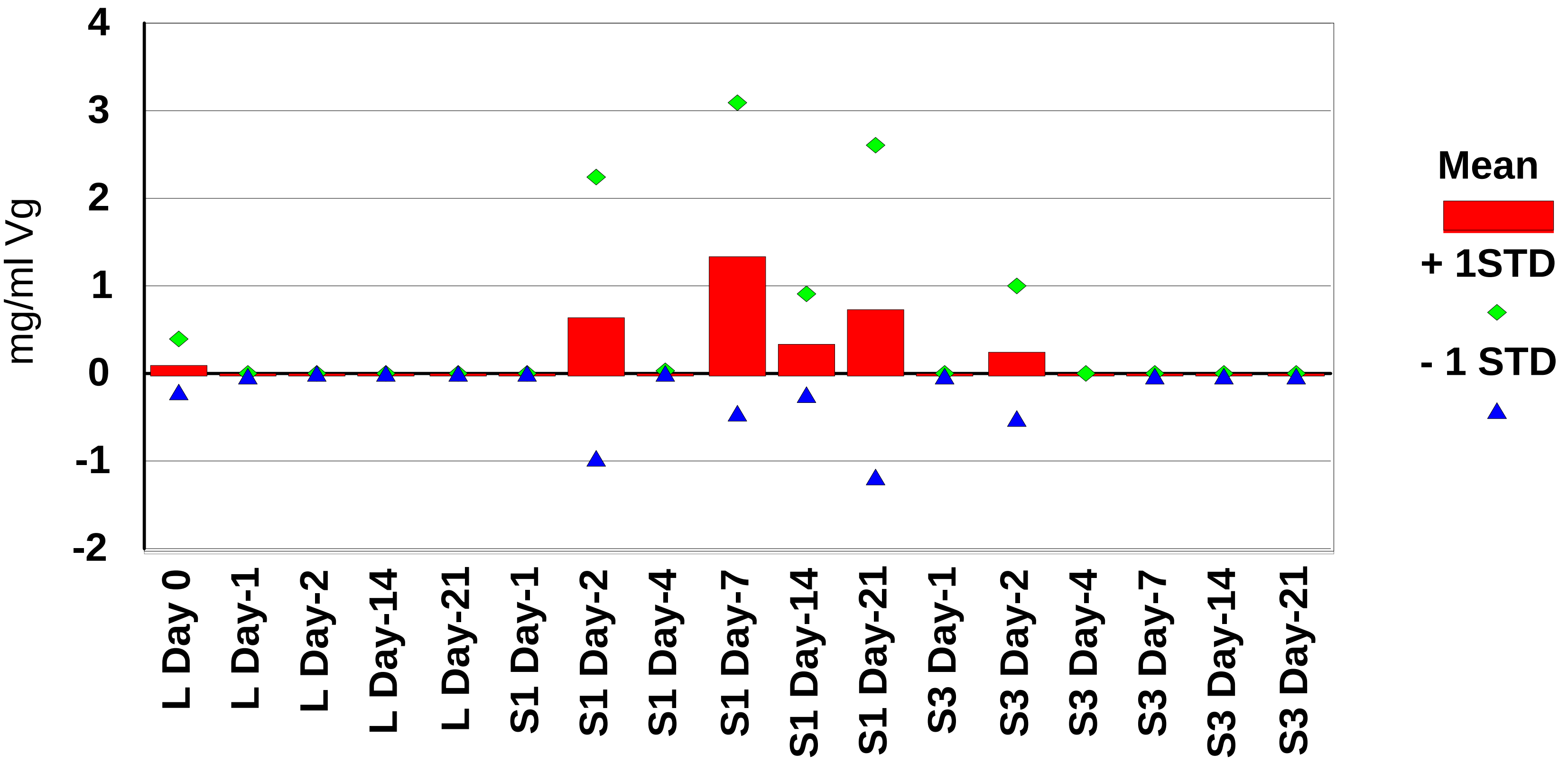
- Field – Fathead minnows and pearl dace
  - Vitellogenin (Vg)
    - Texas - Pecan Creek - Source-biased WWTP
    - University of Kansas – Mesocosms
    - Canada - Whole lake ecosystem study
    - Lake Hartwell – capped sediment study
    - New Mexico - Region 6 - Source-biased WWTP
    - Neuse River Basin - EDC integration into NERL multimedia model development

On Site Wastewater Exposures – Pecan Cr. TX, Vg Gene Expression Levels

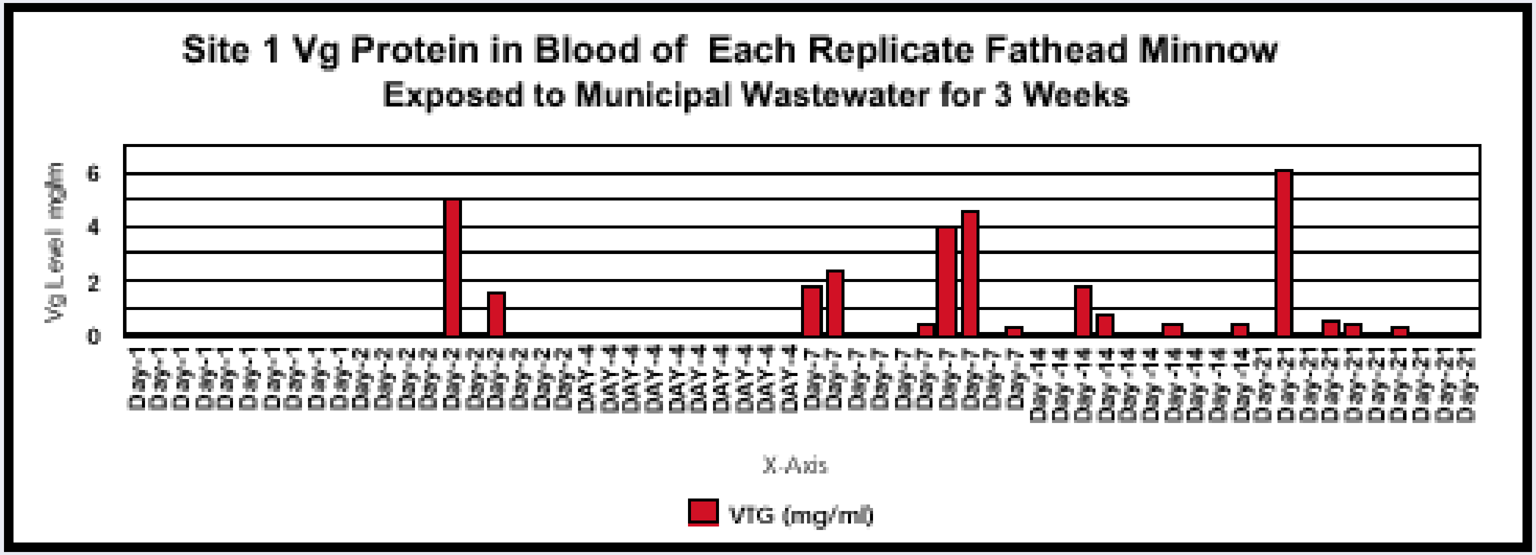


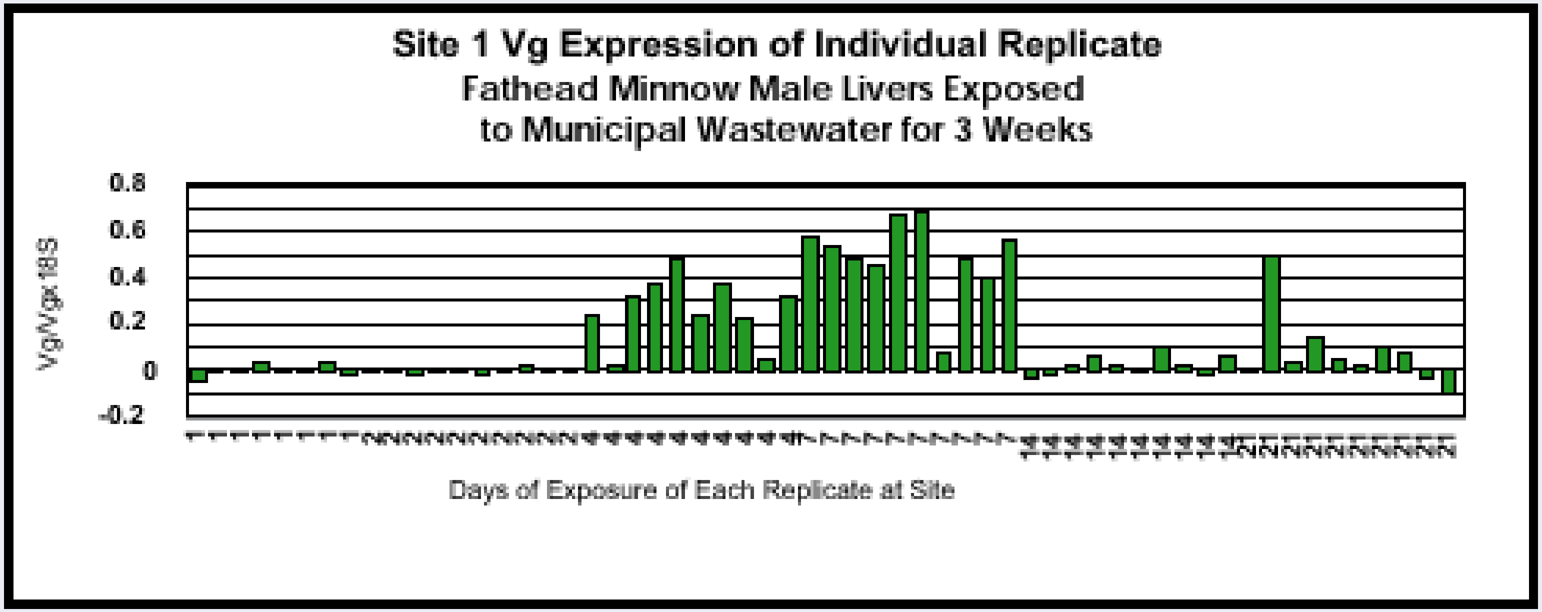
On Site Wastewater cont. Vg Plasma Levels

Vg Plasma Levels in Fathead Minnows



L = Lab Control, S1 = Site Closest to Effluent, S3 = Farthest from Effluent


Site 1 Vg Protein in Blood of Each Replicate Fathead Minnow
Exposed to Municipal Wastewater for 3 Weeks
Vg Level mg/m
6
4
2
0
X-Axis
VTG (mg/ml)


Site 1 Vg Expression of Individual Replicate
Fathead Minnow Male Livers Exposed
to Municipal Wastewater for 3 Weeks
Vg/Vgx18S
0.8
0.6
0.4
0.2
0
-0.2
Days of Exposure of Each Replicate at Site

Canadian Experimental Lakes Area

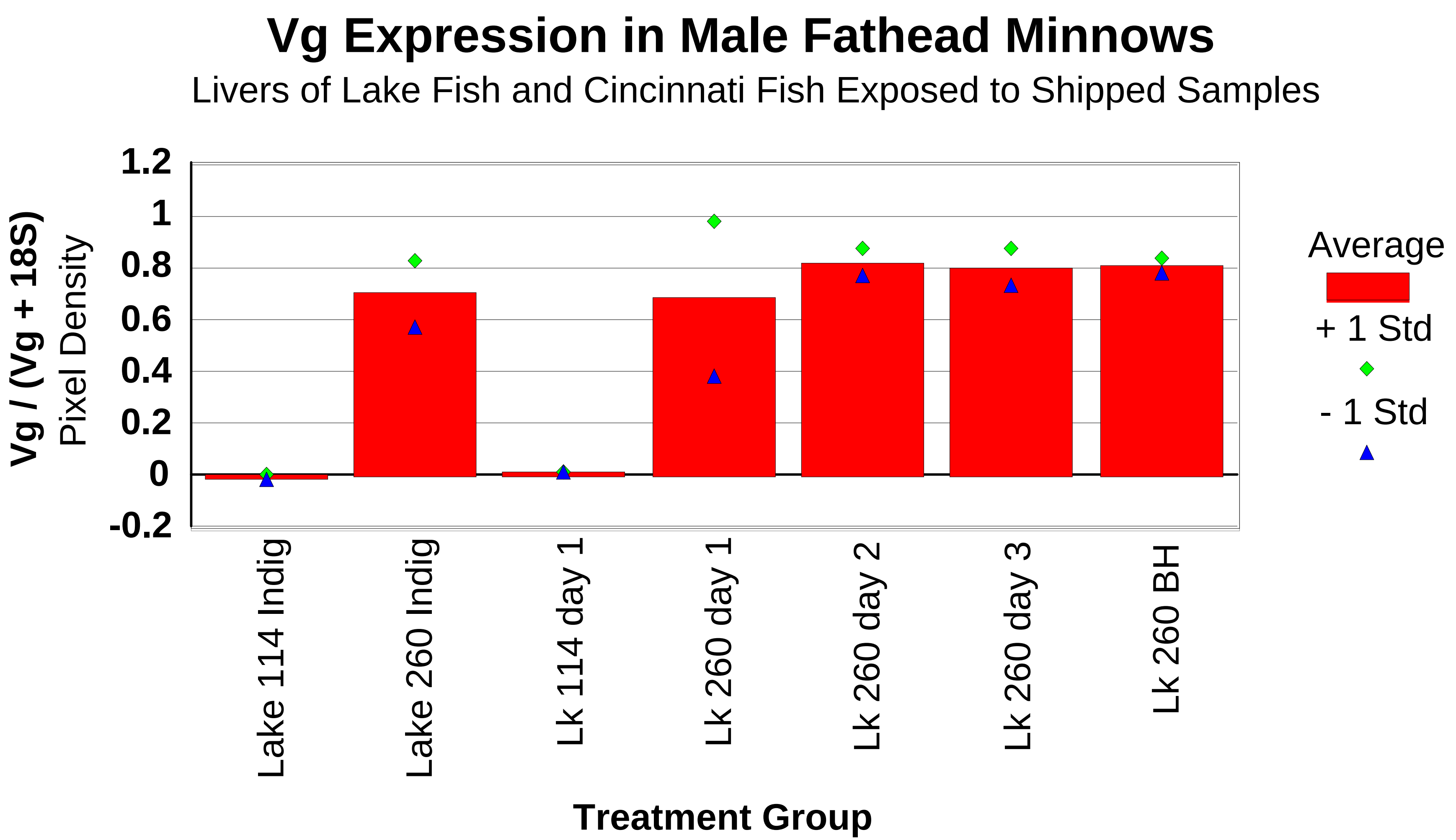
Single gene expression – Vg – Field

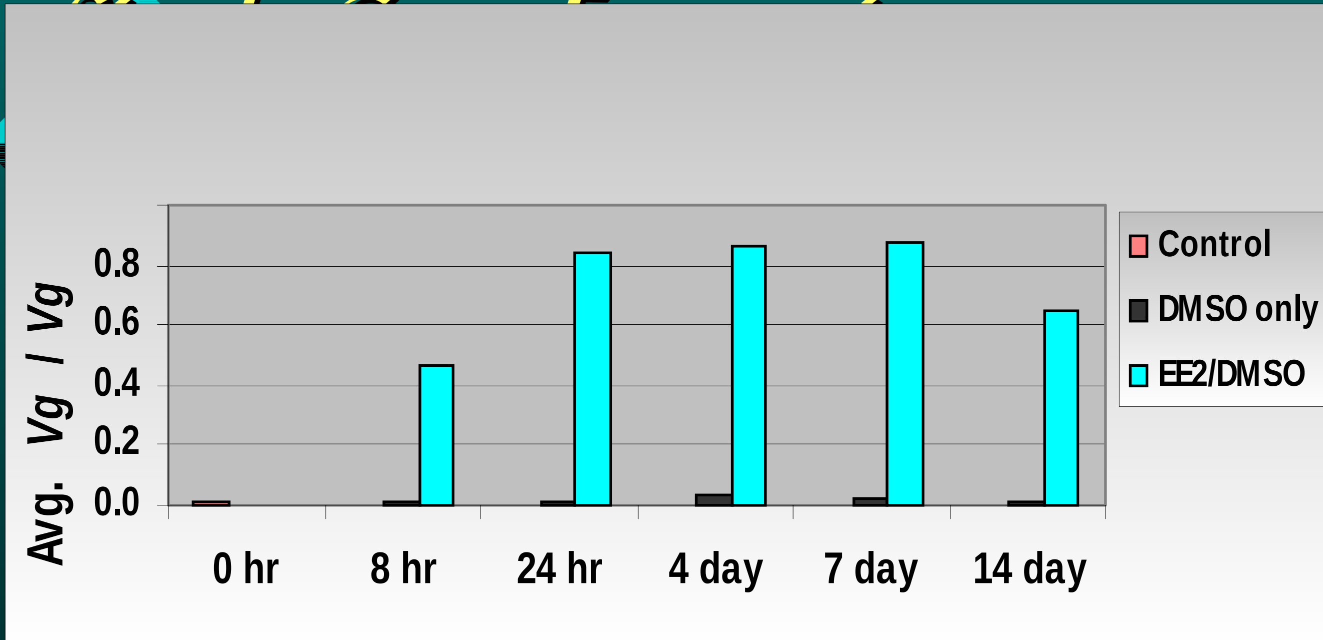
- Canadian Experimental Lakes Area

dy

ut

Other EDC Studies – Whole Lake Exposures



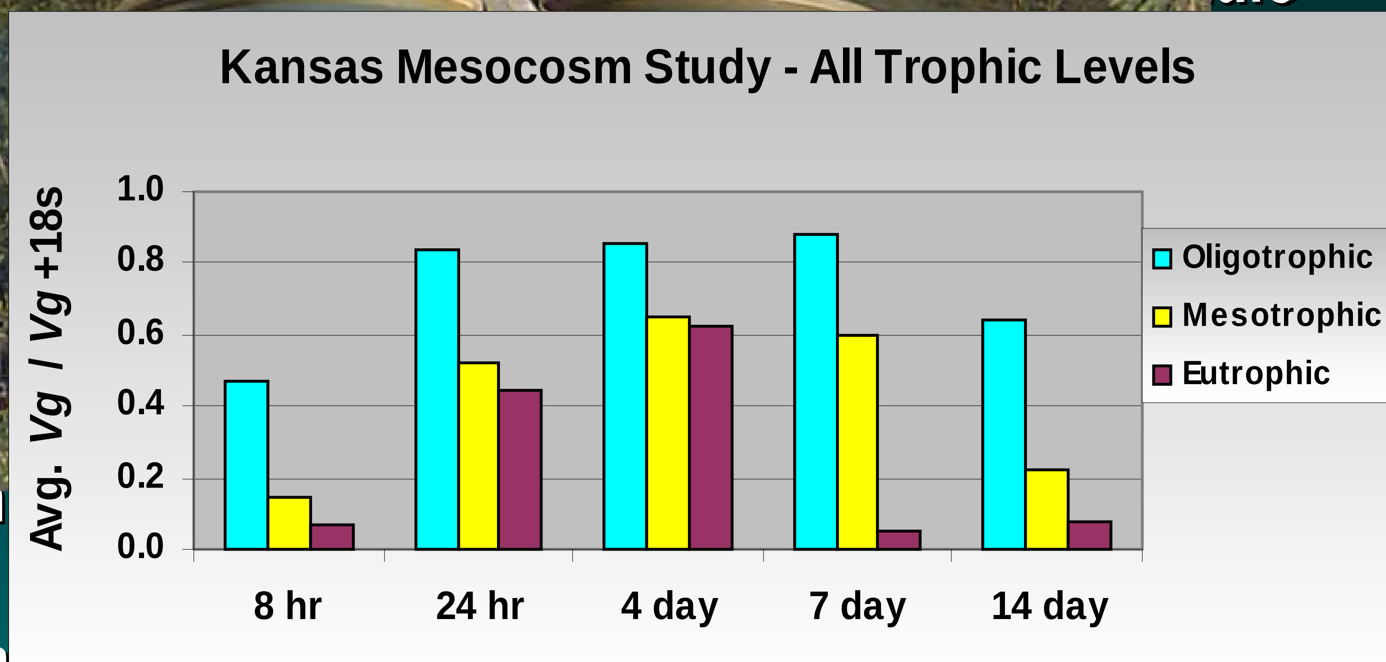


Area

ale

– Eth

– 8 h

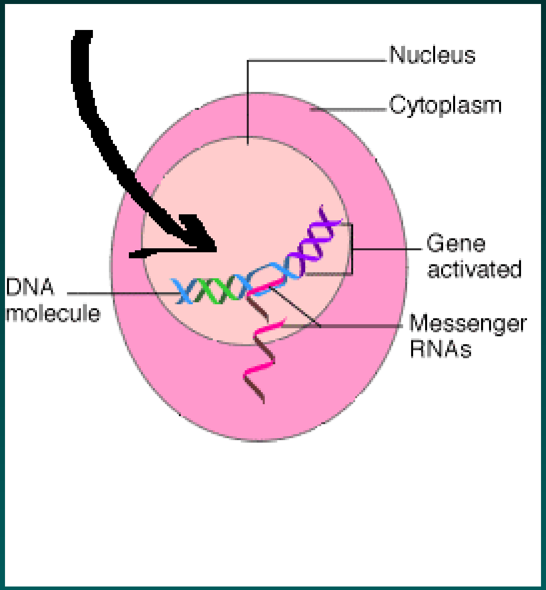


Current Studies

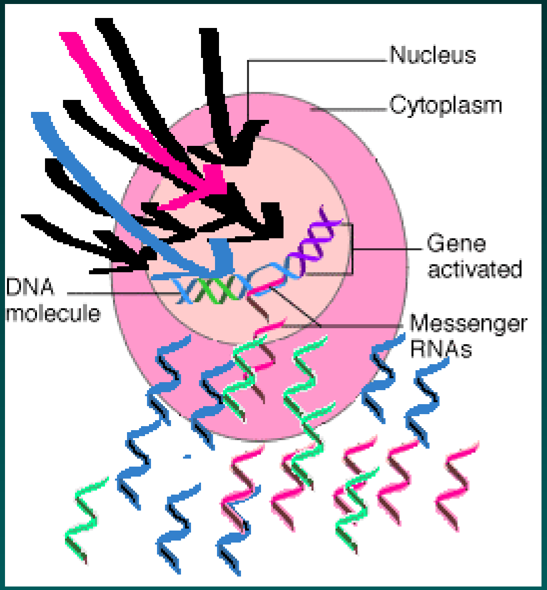
- Single Gene Indicator Studies
  - Laboratory - Adult male and larvae fathead minnow
    - Vitellogenin (*Vg*)
      - Estrogens (estradiol, ethynylestradiol, DEHP)
    - Cytochrome P450IA1
      - PAH's, PCB's
    - Metallothionein
      - Metals (Cd, Cu)

Two Study Themes

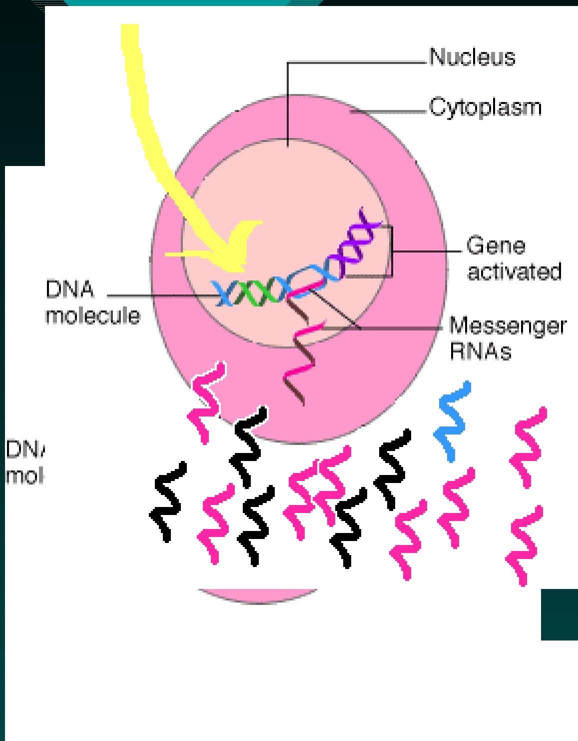
- Single stressor / single known gene

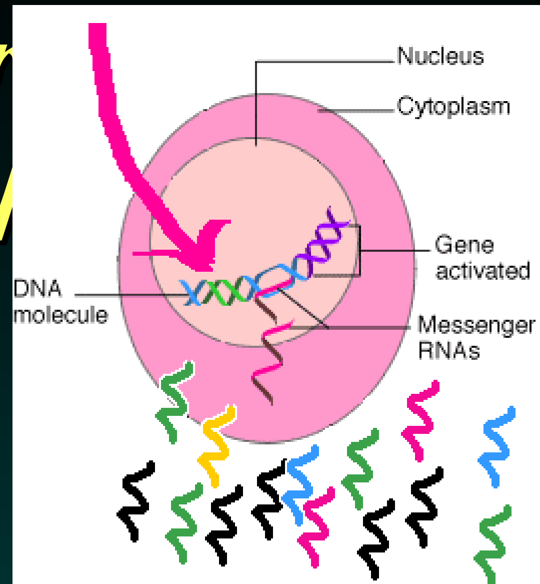


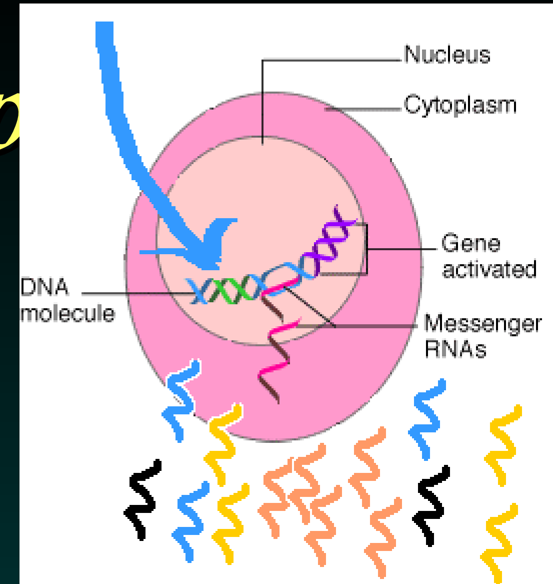
- Multiple stressors / multiple genes

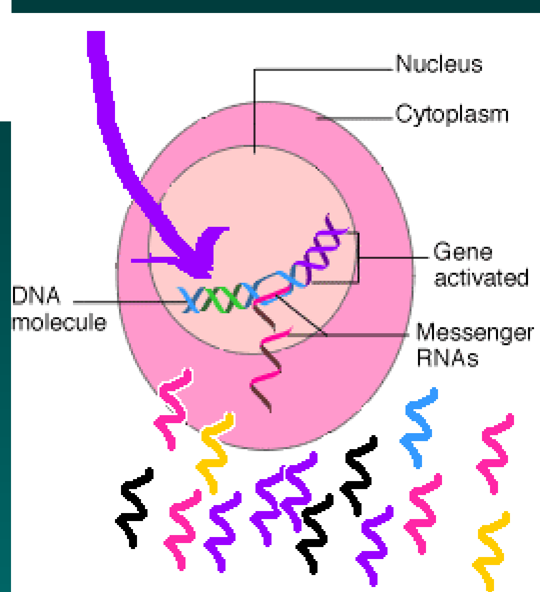


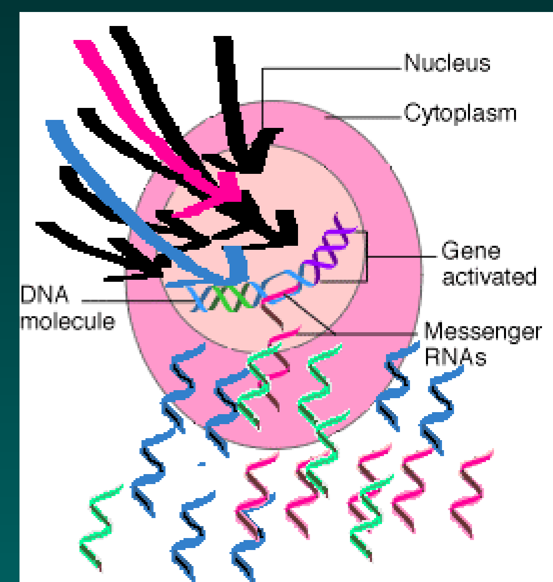
Multiple Str … ltip …





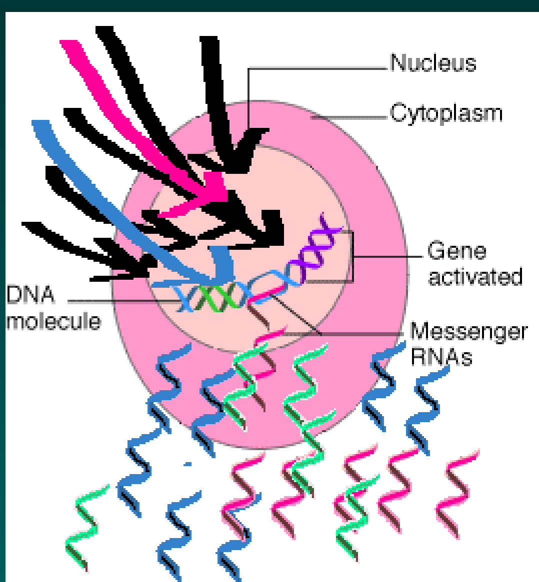




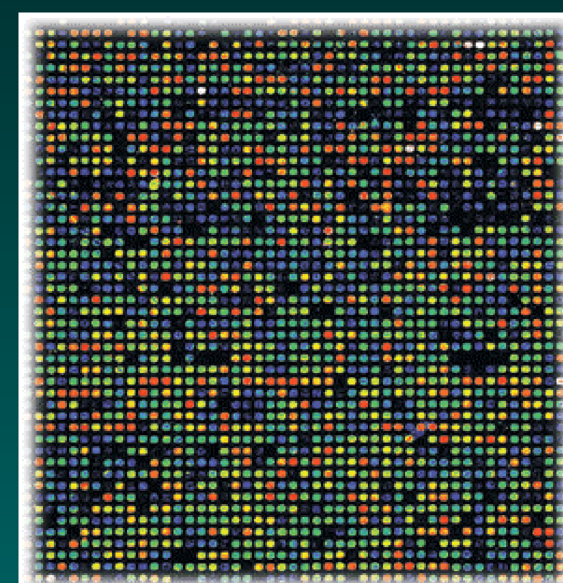


Multiple Gene Expression
Laboratory Studies

- DNA microarrays
  - Glass slide with DNA spots
    - Detect thousands of changes in gene expression relative to controls or other exposures

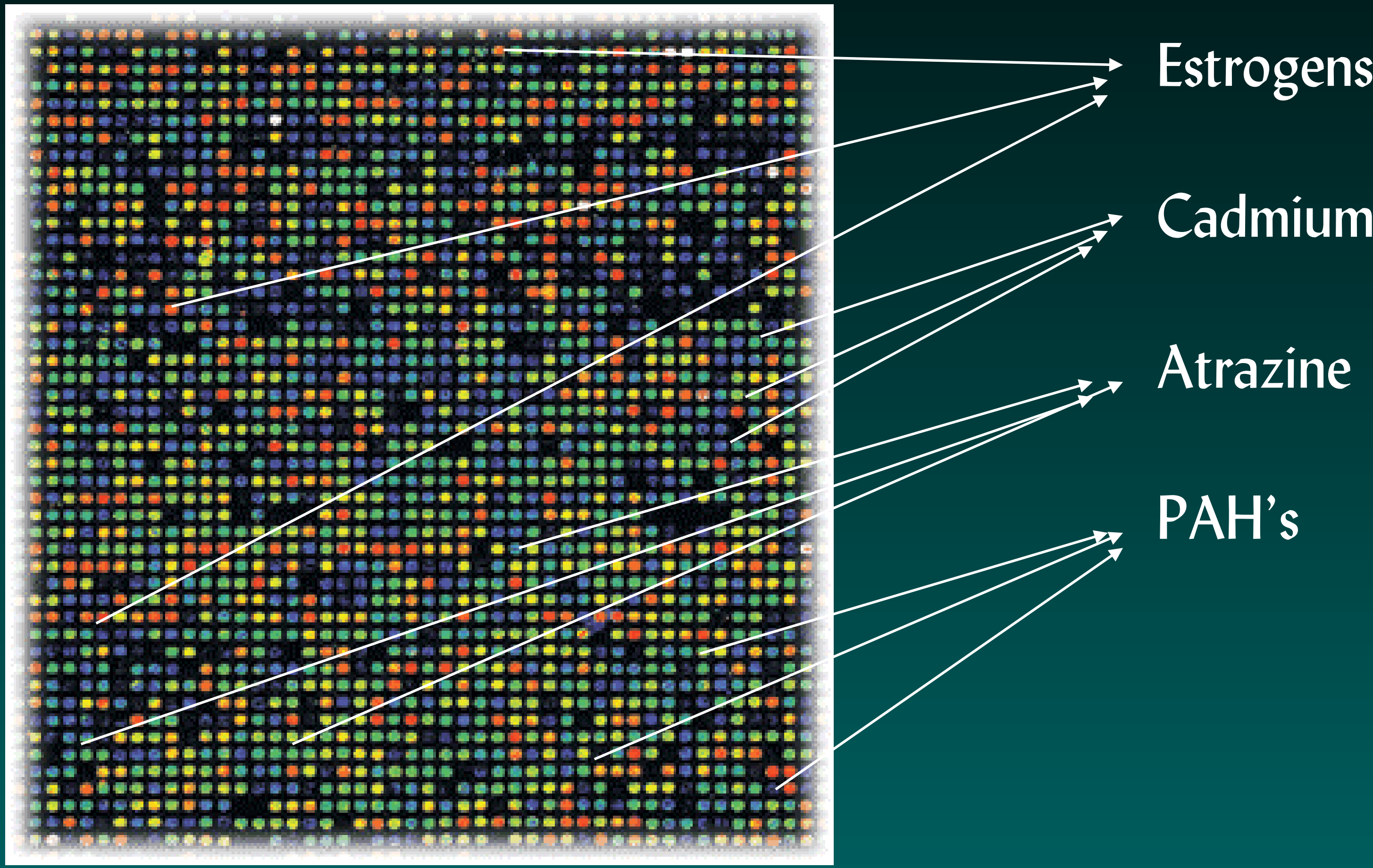


Studied with--



DNA Microarray

Multiple Gene Expression
Laboratory Studies



DNA Microarray

Multiple Stressor Diagnostics

Multiple Gene Studies

- Stressors
  - EDCs
    - Estrogens
    - Androgens
    - Thyromimetics
    - PAHs
    - Metals
  - Pesticides
    - Atrazine
    - Alachlor
  - Pharmaceuticals
    - Fluoroquinolones

EERD Biological Indicators

Where to from here-

- More exposure questions out there than the ones we're asking
- Commitment to technology transfer – demos in your backyard
- Core molecular capabilities are unsurpassed in ORD
- Field capabilities are unique

Assessment of Estrogenic Potential & Bioavailability of PCBs in Lake Hartwell Water and Sediment

James M. Lazorchak[1,] David Lattier[1], Mark E. Smith[2], Barry Wiechman[3], Dan Williams[2,] Richard C. Brenner[4], Victor S. Magar[5],

[1] USEPA NERL, Cincinnati, OH

[2] SoBran Inc. c/o USEPA NERL, Cincinnati, OH

[3] PAI, c/o USEPA NERL, Cincinnati, OH

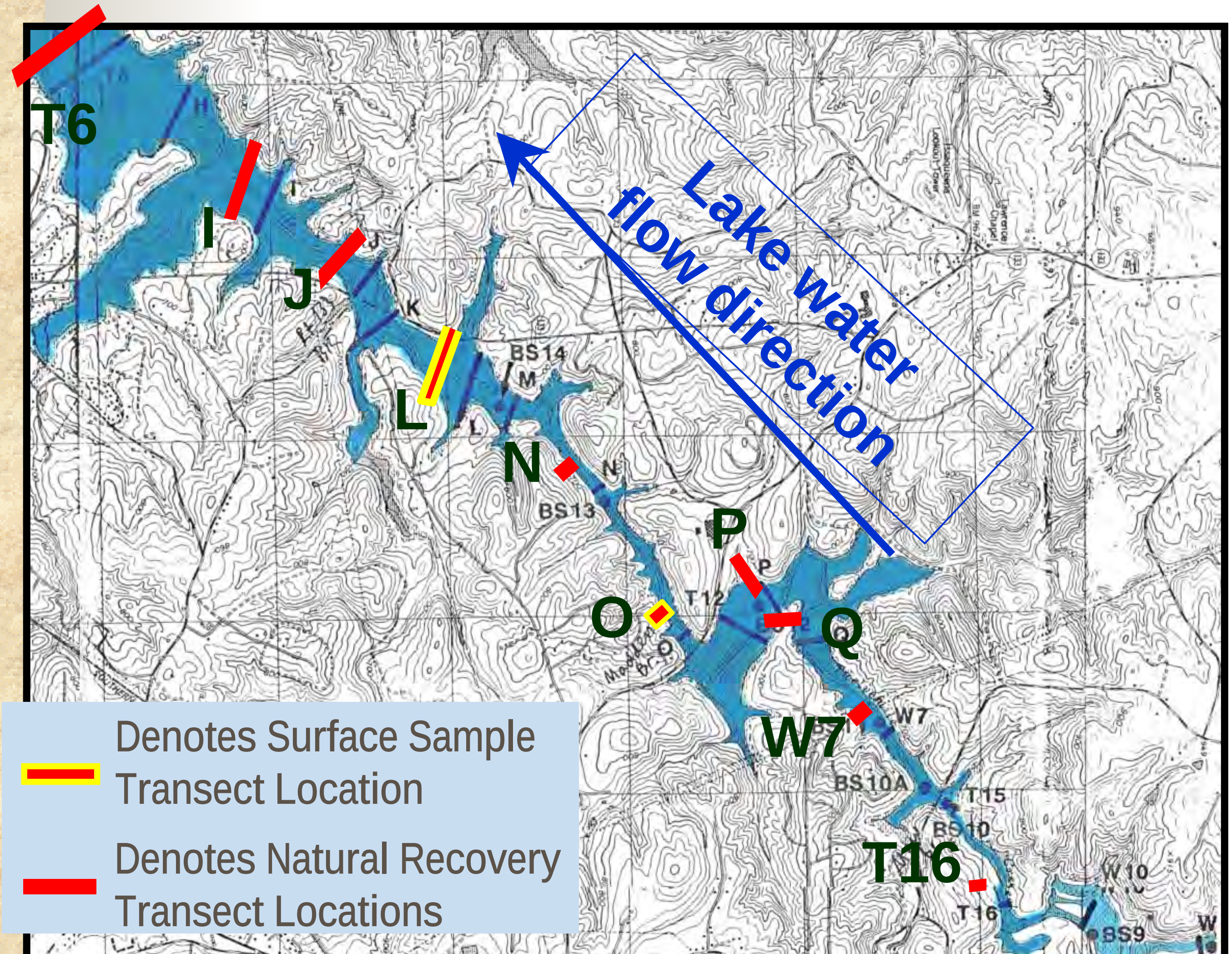
[4]U.S. EPA, NRMRL, Cincinnati, OH

[5]Battelle, Columbus, OH

Objectives

- Determine if PCBs in Lake Hartwell water are estrogenic to male fathead minnows and/or Fry or if sediments are estrogenic to larval fathead minnows.
- Determine if Lake Hartwell sediments are toxic to freshwater amphipods and/or fathead minnow embryos.
- Determine if PCBs in water and sediments of Lake Hartwell are bioavailable.

Lake Hartwell Site Map



- Contaminated sediment sample locations: Transects L & O
- Locations matched USEPA and natural recovery transects
- Uncontaminated water collected from the Keowee River

Approach – Water Samples

- 4 Water column samples:
  * Bkg = Keowee River
  * 3 Transects = T-O, T-L, T-I

- 4 Controls were used:
  * 2 positive controls of a 50:50 mixture of Aroclors 1242 & 1254 at 10 and 100 ng/L in DMSO
  * 2 laboratory water controls of moderately hard water with and without DMSO at 3.74 *u*g/L.

Approach – Water Samples (Cont.)

- Each water sample was tested by exposing 5 individual 11-13 month old adult male fathead minnows for 24-hrs.

Approach - Sediment

- Nine Sediment samples
    - * Bkg = Keowee River
    - * Transects = 3 T-O, 3 T-L, 2 T-I

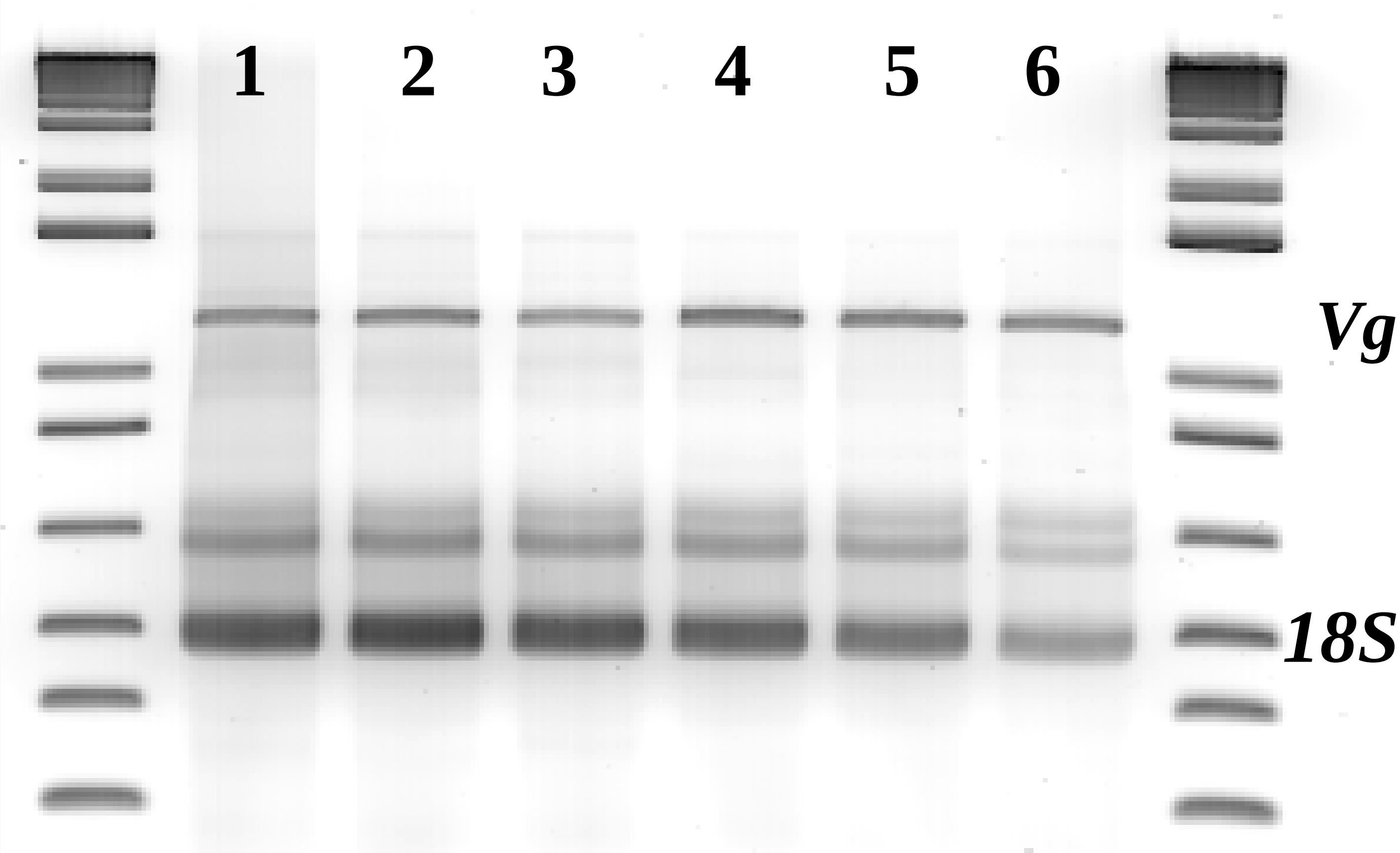
- 4 Control sediments
    - * Sand Control Sediment – Grade 30
    - * PCB Positive Sand control (10 ng/L of 50:50 Aroclors 1242 and 1254)
    - * 2 EE2 Positive Sand Controls (10 & 20 ng/L Ethynylestradiol)

Approach – Sediment (Cont.)

- Each sediment was tested using a total of 160 24- to 48-hr old fathead minnow eggs for 7 days and collected for Vg analyses after they hatched.

- Each sediment was tested using pooled 120 10-day old amphipods for 7days.

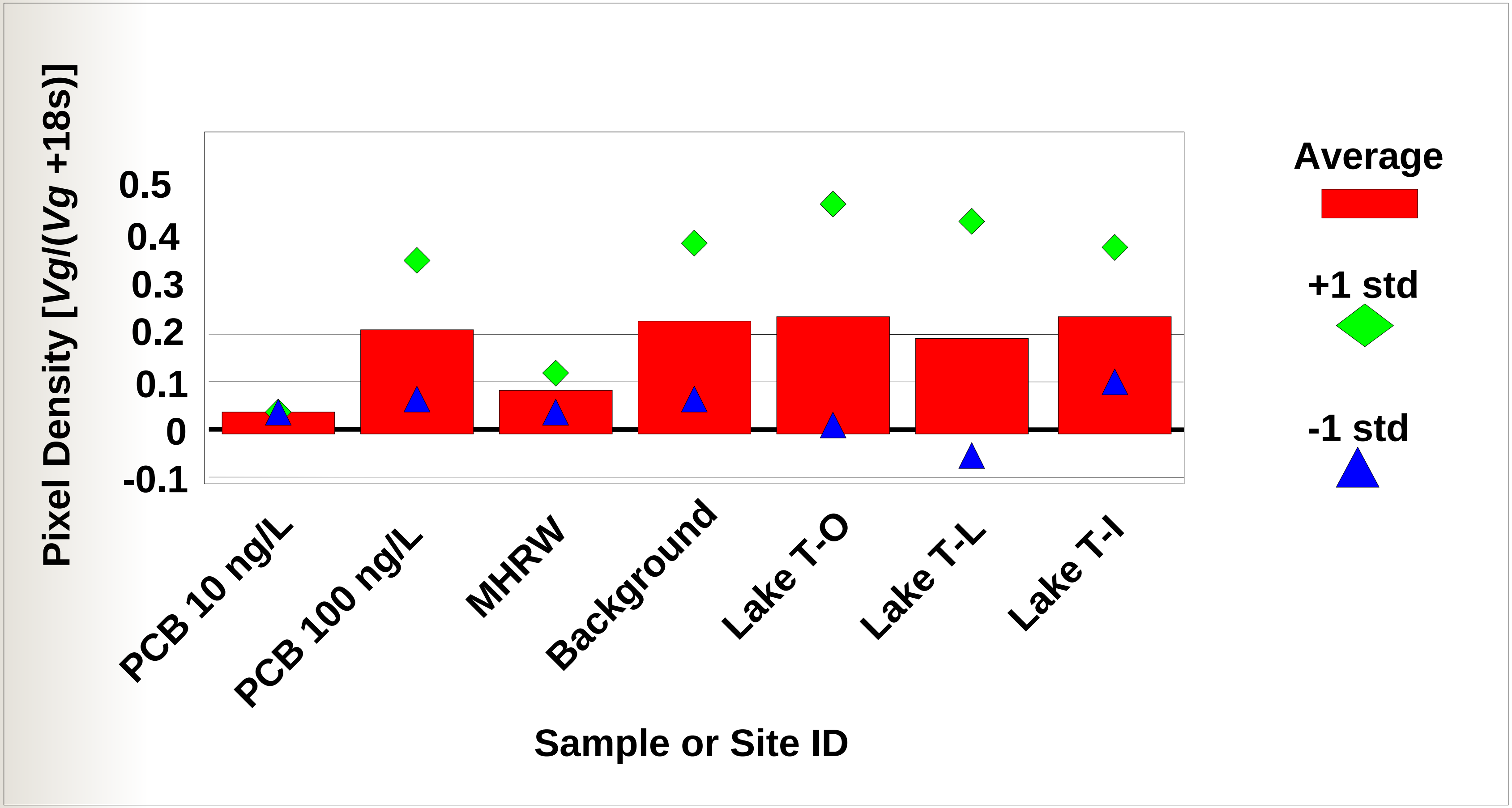
Example on How Gene Expression is Quantified



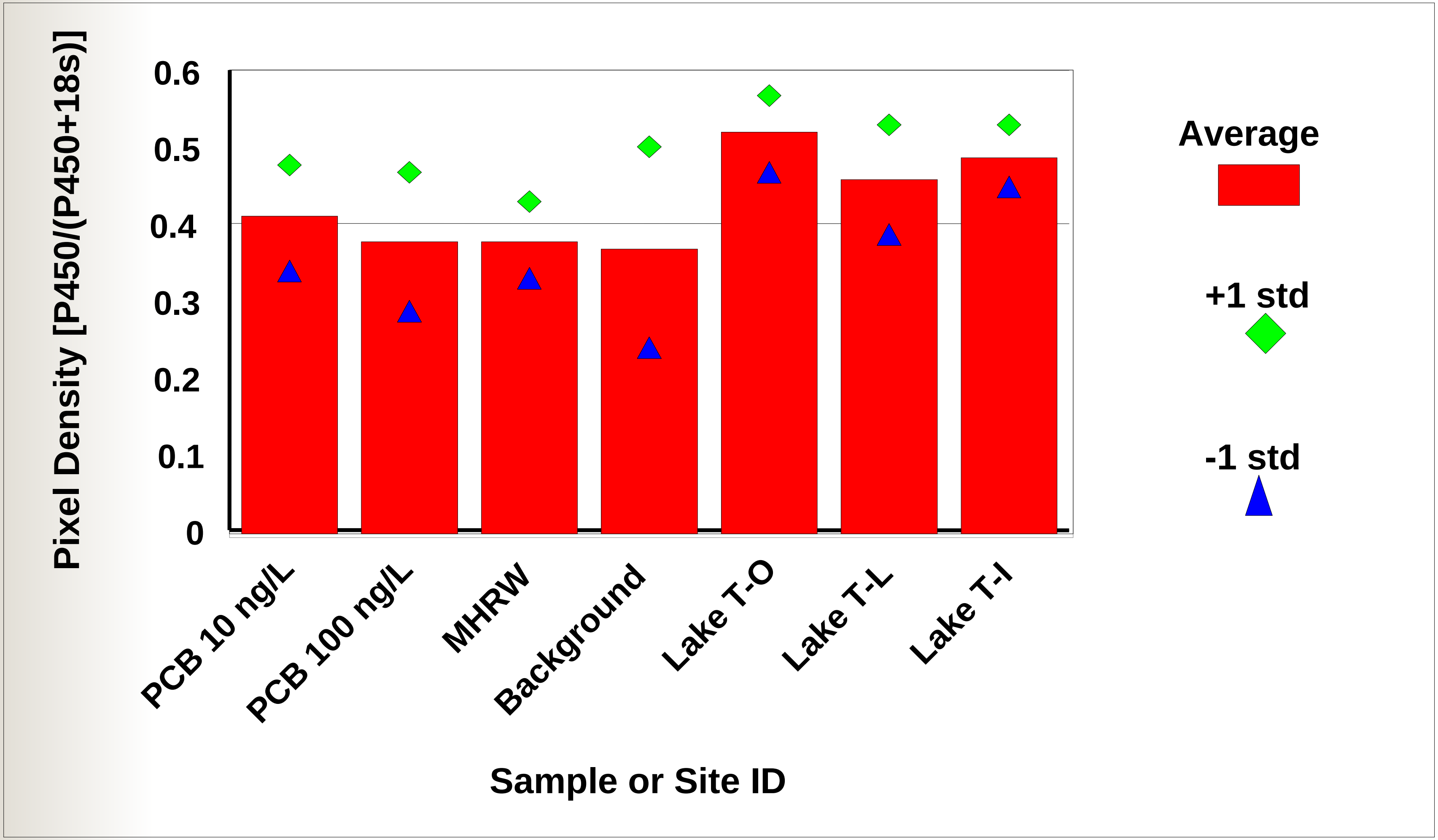
Pixel Density

Fathead Minnow Fry Data	
Name	**Volume**
RECT-1-Vg	15419135.47
RECT-1-18s	61092632.94
RECT-2-Vg	14860688.45
RECT-2-18s	67114894.24
RECT-3-Vg	11051396.38
RECT-3-18s	59201253.85
RECT-4-Vg	17506216.8
RECT-4-18s	52326040.86
RECT-5-Vg	15044467.94
RECT-5-18s	41589259.53
RECT-6-Vg	13696792.53
RECT-6-18s	26349086.86

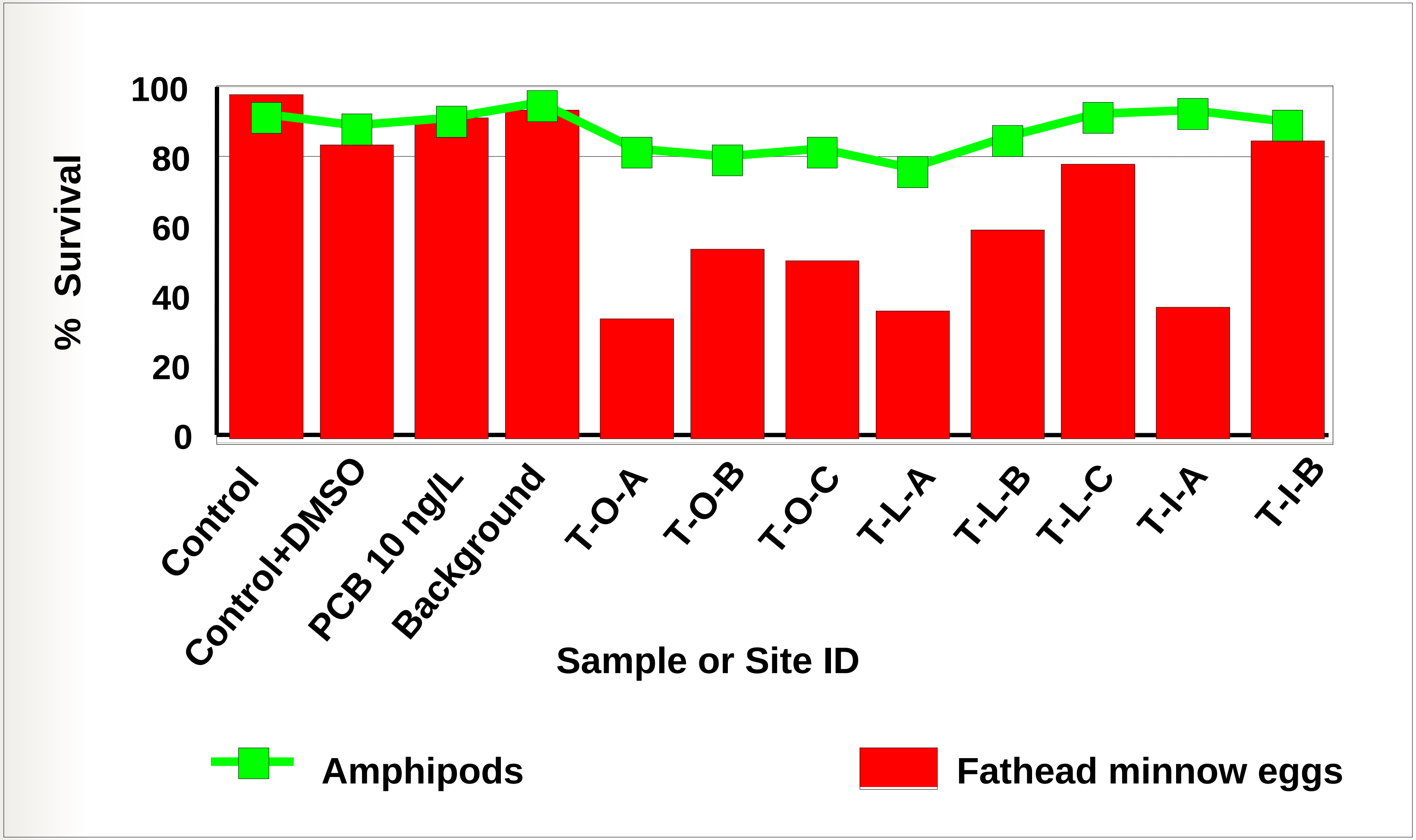
Vg Results in Male Fathead Minnow Livers Exposed to Lake Hartwell Water Samples



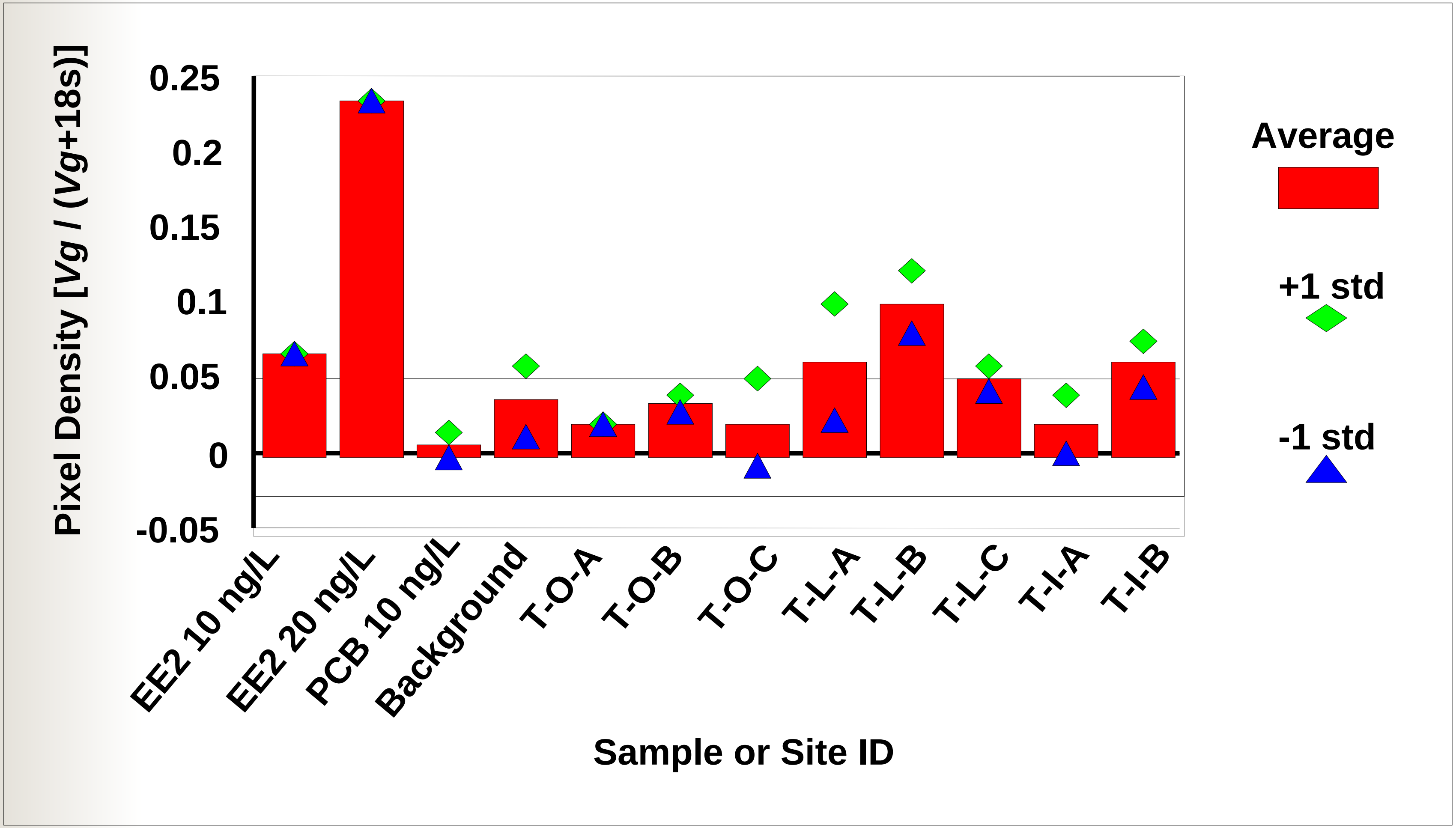
P450 Results in Male Fathead Minnow Liver Exposed to Lake Hartwell Water Samples



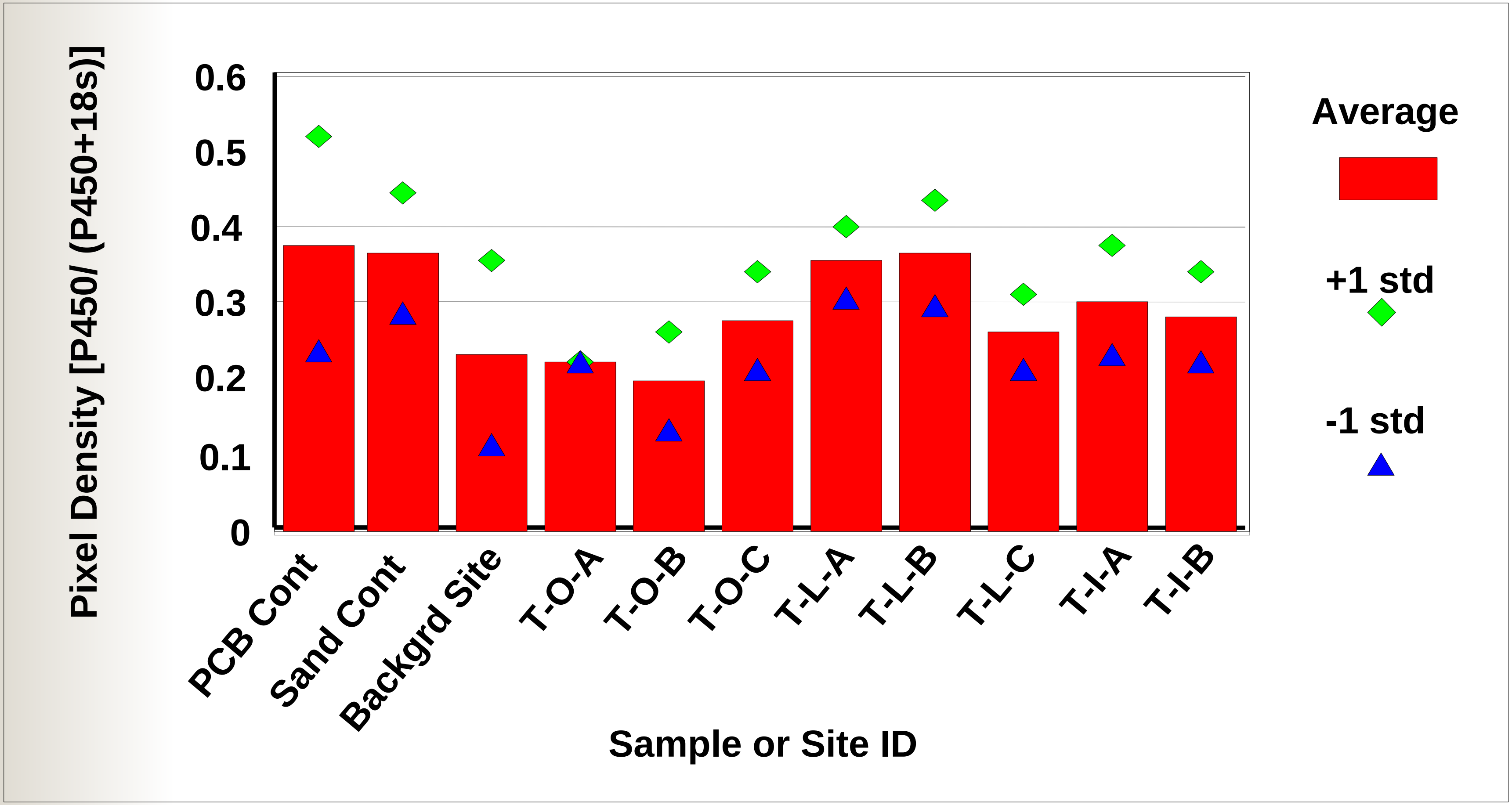
Survival Results of Amphipods and Fathead Minnow Eggs in Lake Hartwell Sediments



Vg Results In Fathead Minnow Eggs Exposed to Lake Hartwell Sediment Samples



P450 Results In Fathead Minnow Eggs Exposed to Lake Hartwell Sediment Samples



Observations - Water

- No toxicity to fathead minnow fry or adults was found in water samples.
- Vitellogenin gene (*Vg*) expression was found in male fathead minnows in all Lake Hartwell water samples.
- No *Vg* Gene Expression was detected in Fry – (Exposure may need to be >48hr)

Observations – Water (Cont.)

- *Vg* Gene expression in Lake Hartwell Water samples was >10 ng/L < 100 ng/L 50:50 Aroclors 1242/1254.

- P450 Gene expression was found in all water samples and was $\geq$100 ng/L PCB.

Observations – Sediment

- Sediment samples from T-O, T-L, and T-I-A were toxic to fathead minnow eggs – UN-NH3
- Vitellogenin gene expression in FHM eggs exposed to T-L-B, T-L-C, and T-I-B was higher than Sand Control or Keowee River sediments.
- Vitellogenin gene expression was > 10 ng/L EE2. Previous studies have detected *Vg* expression as low as 5 ng/L EE2 in FHM eggs and 2 ng/L EE2 in adults.

Observations – Sediment (Cont.)

- P450 expression in Lake Hartwell sediments was not different than control samples.
- P450 gene expression in Fathead Minnows was not a good indicator of PCB bioavailability.
- Working on new genes.

Future Directions & Projects

- Continue EDC studies on Lake Hartwell

  * Repeat *Vg* Water & Sediment tests

  * Caged *Vg* Fish Studies

  * Look at *Vg* Indigenous related species
- National Screening Assessment of 50 Municipal Effluents
- CAFO Screening Assessment in Ohio

An Engineering Approach to Abatement of Estrogenic EDCs in Wastewater

Paul McCauley

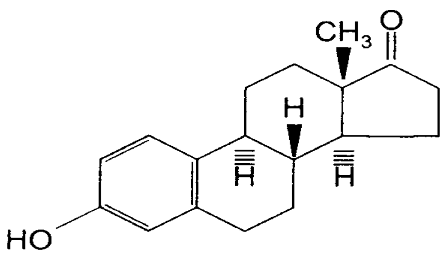
Who is involved?

Paul McCauley	Chemist	U.S. EPA
Greg Sayles	Chemical Engineer	U.S. EPA
Barry Austern	Chemist	U.S. EPA
Marc Mills	Environmental Engineer	U.S. EPA
Carolyn Acheson	Chemical Engineer	U.S. EPA
Eric Kleiner	Environmental Engineer	U.S. EPA
Richard Brenner	Environmental Engineer	U.S. EPA
James Lazorchak	Ecotoxicologist	U.S.EPA
Alan Zaffiro	Analytical Chemist	IT Corporation
George Sorial	Chemical Engineer	Univ. Cinti.
Mar Esperanza	Chemical Engineer	Univ. Cinti.
Cindy Boardman	Biologist	Univ. Cinti.
Makram Suidan	Environmental Engineer	Univ. Cinti.

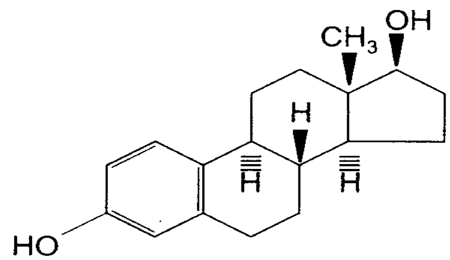
The Problem

- Publicly Owned Treatment Works (POTW) Discharges appear to have Estrogenic Effects on Several Species of Fish
- This effect appears to be mediated through the estrogen receptor
- Compounds Suspected of these Estrogenic effects include Estrogens and there metabolites and Alkylphenols, there ethoxylates and metabolites

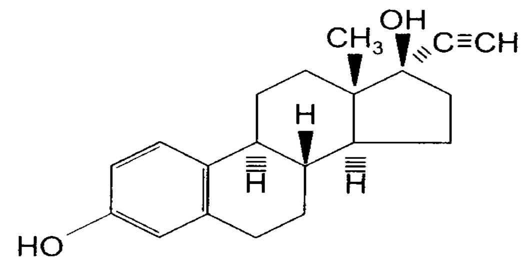
Estrogens and Androgens



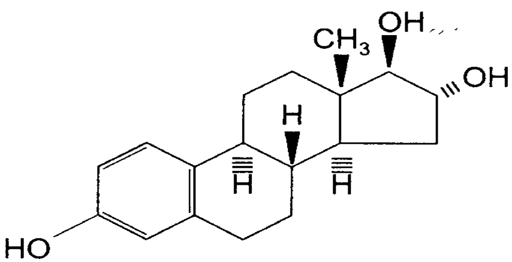
Estrone



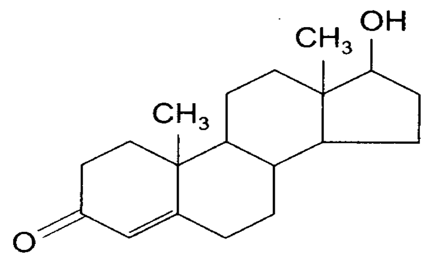
17-β-Estradiol



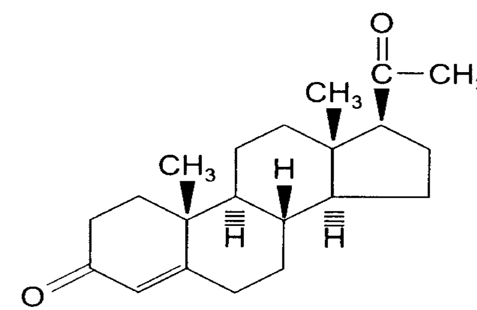
17-α-Ethynylestradiol



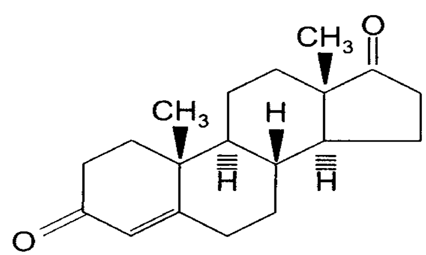
Estriol



Testosterone

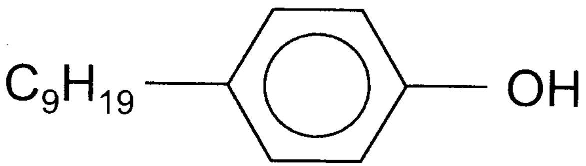


Progesterone

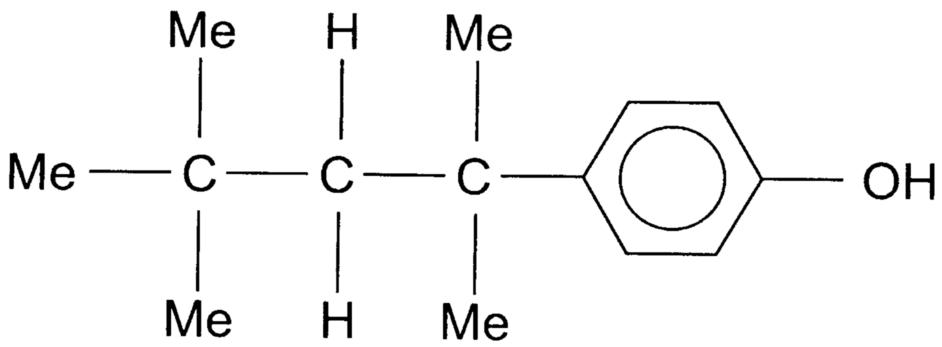


Androstenedione

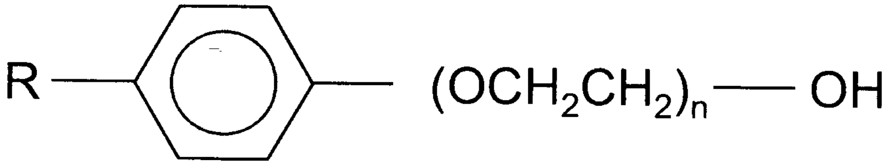
Alkylphenols



4-nonylphenol (NP)



4-*tertiary*-octylphenol (OP)



R=C_9H_{19}, nonylphenol ethoxylates (NPEO)
R=C_8H_{17}, octylphenol ethoxylates (OPEO)

Long Term Objectives

- 1 To determine the fate of Estrogenic EDCs (including Estrogens, there metabolites; Alkylphenols, there ethoxylate esters and metabolites) during wastewater treatment
- 2 To fashion an engineering solution to Estrogenic EDC discharge in wastewater and sludge.

Basic Approach

- Develop assays for the suspect agents
- Construct pilot scale wastewater treatment systems to model metabolic pathways.
- Assay samples from selected and representative POTWs to determine if the pilot scale system is modeling POTWs
- Optimize pilot scale wastewater treatment systems for treating estrogenic EDCs
- Make recommendations for improving EDC treatment in POTWs

The Proposed Assays

- Steroid analysis using solid phase extraction and GC/mass spectrometry
- Nonylphenol ethoxylate analysis using solid phase extraction and normal phase HPLC.
- Fathead minnow using estrogen receptor mediated induction of vitellogenin by measuring messenger RNA assay (NRML)
- Recombinant Yeast assay

Steroid Analysis

- Liquid and Solid fractions shall be separated and analyzed separately.
- Solids shall be extracted
- All fractions will then be concentrated using solid phase extraction (SPE) Extracts from the SPE will be derivatized and analyzed by GC Mass Spectrometry
- Goals for detection thresholds is 1 ng/L for each steroid.

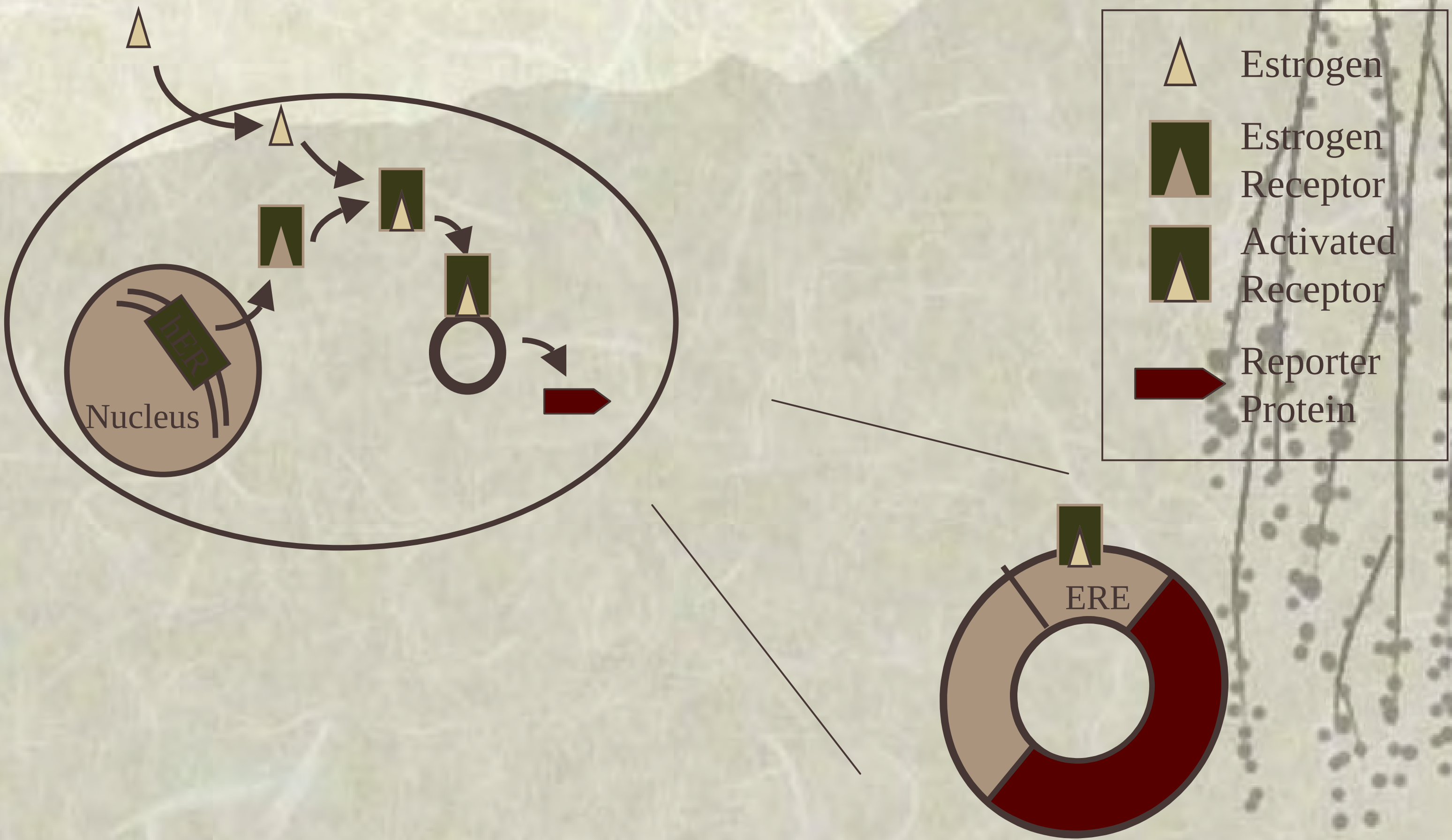
Nonylphenol Ethoxylate Analysis

- Liquid and Solid fractions shall be separated and analyzed separately
- Solids shall be extracted
- All fractions will then be concentrated using solid phase extraction (SPE) Extracts from the SPE will be analyzed by normal phase HPLC
- Goals for detection thresholds is 50 ng/L for each alkylphenol ethoxylates

Messenger RNA Assay

Supply samples to NERL for mRNA (vitellogenin) induction analysis in Fathead Minnow

Recombinant Yeast Assay

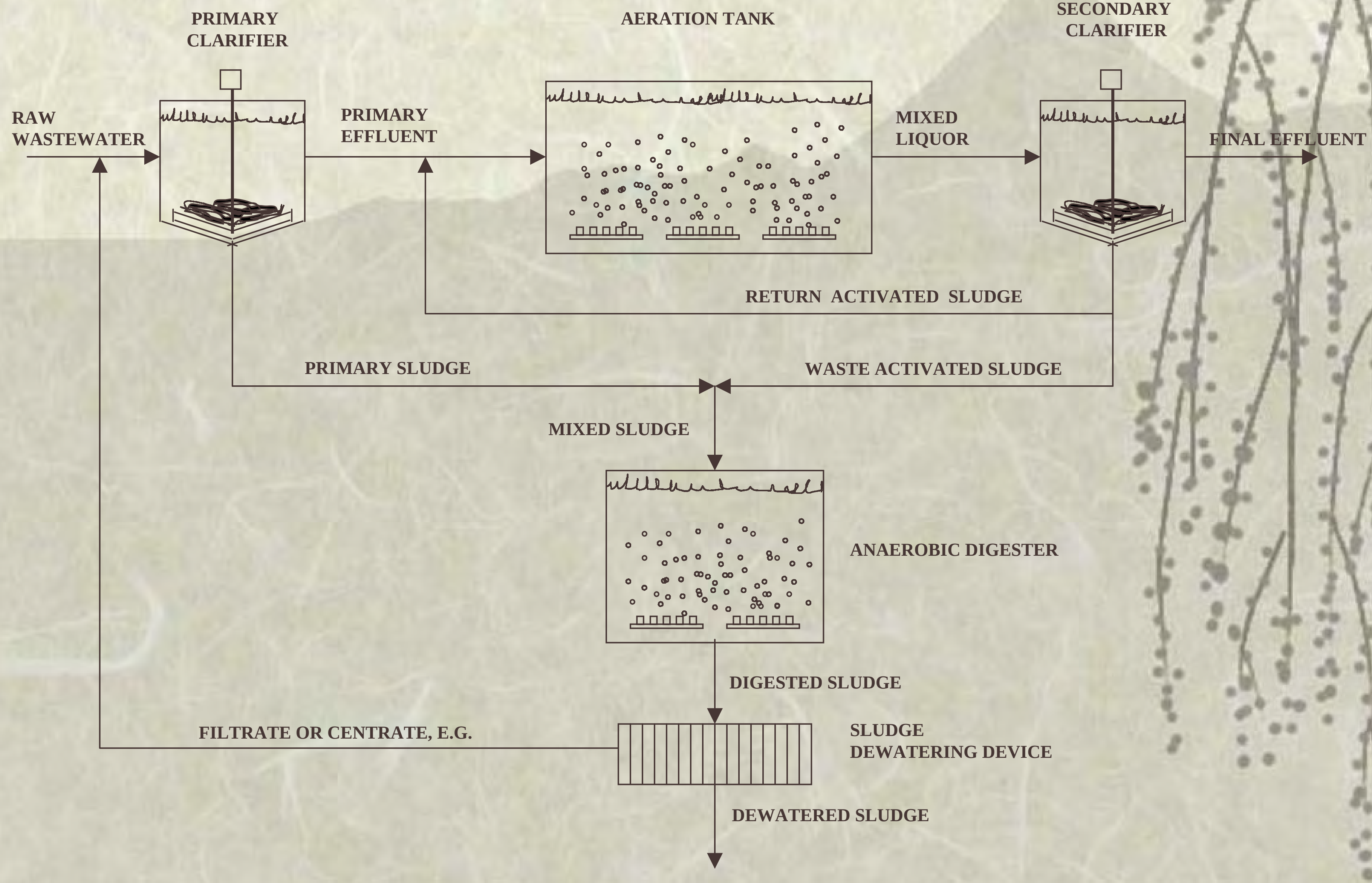


From Routedge and Sumpter, 1996. Environ. Tox and Chem. 15: 241- 248

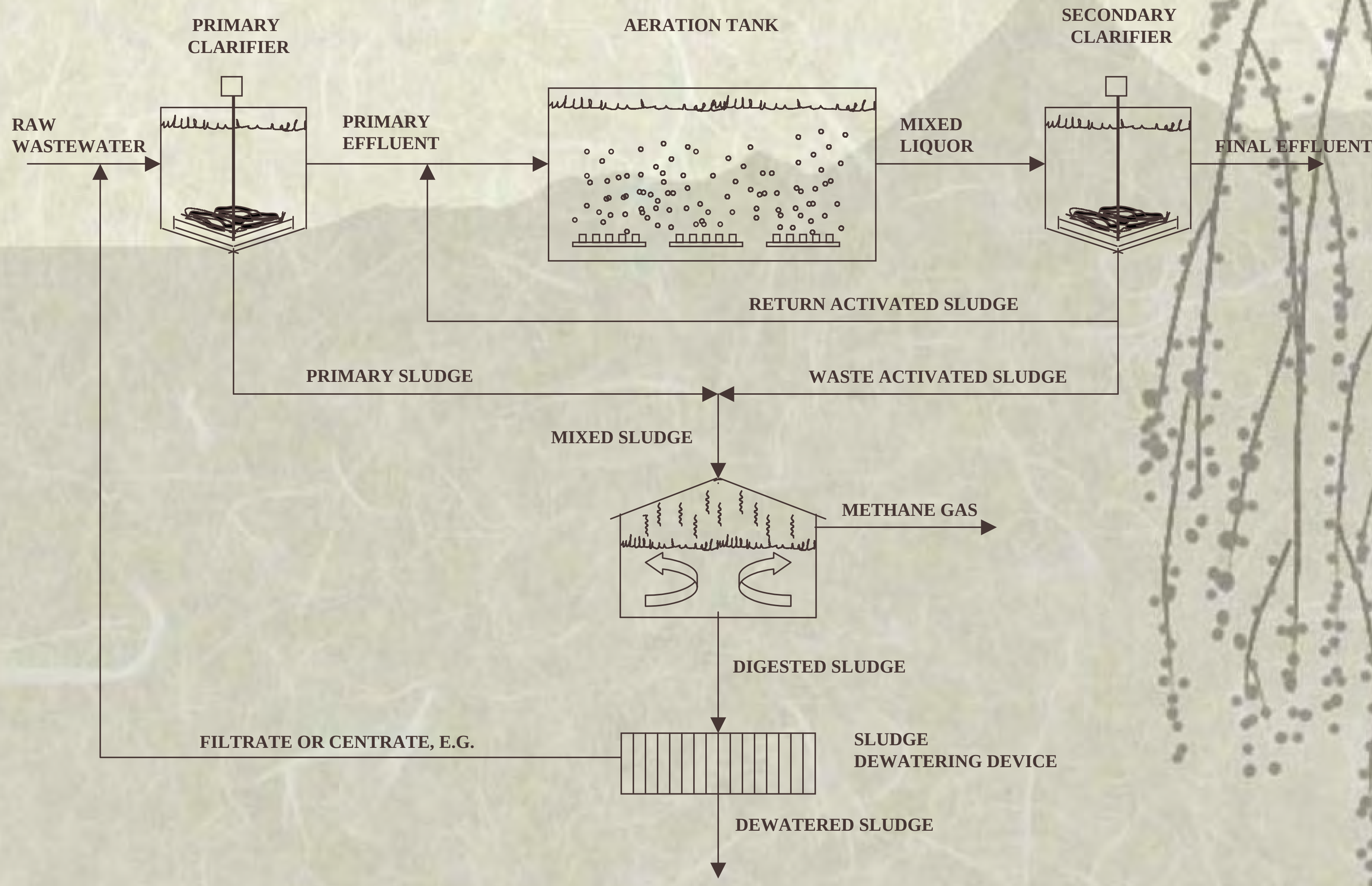
Pilot scale wastewater treatment systems

- EDC Pilot Plant with Aerobic Sludge digestion
- EDC Pilot Plant with Anaerobic Sludge digestion

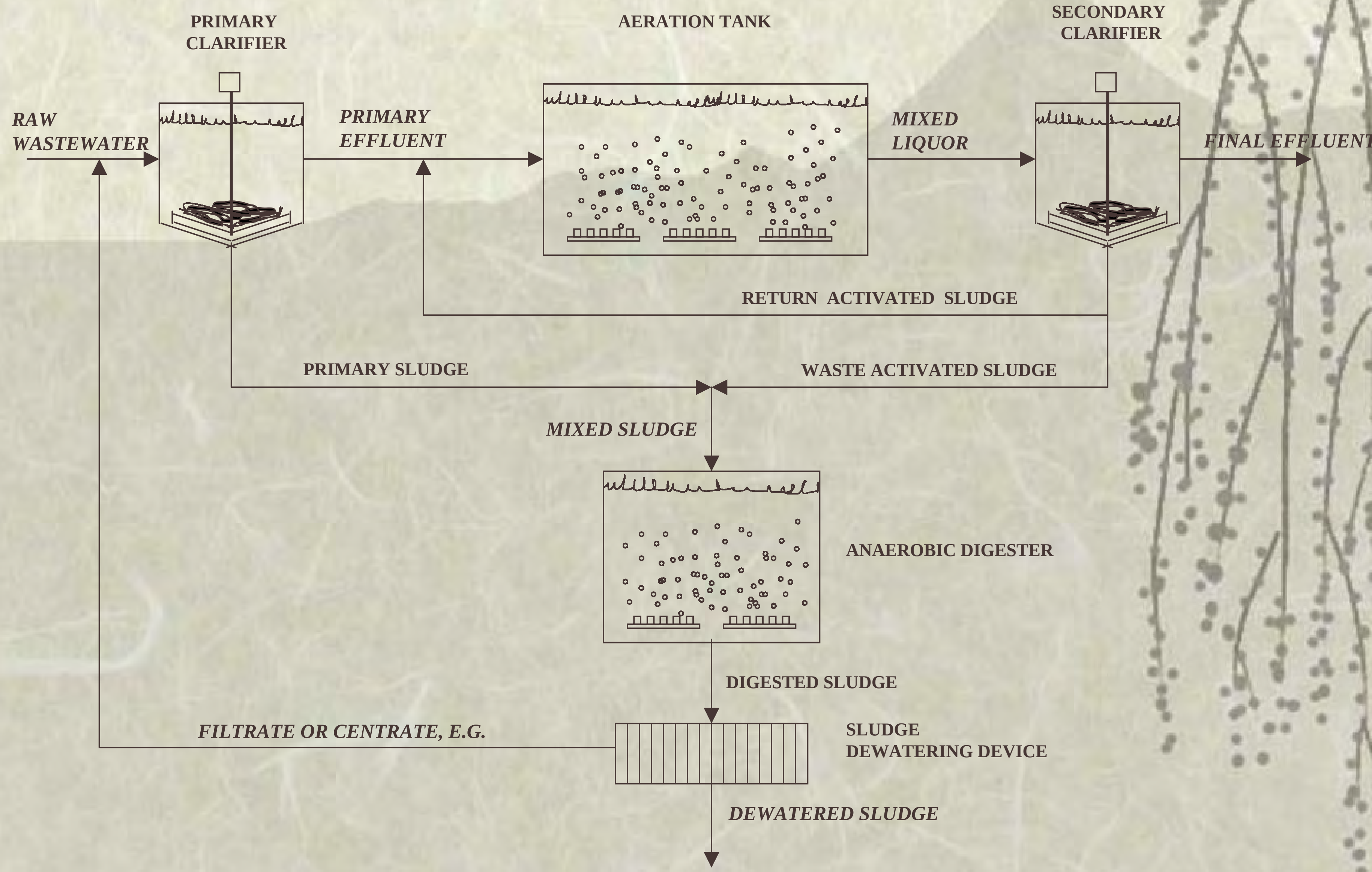
EDC PILOT PLANT FLOWSHEET WITH AEROBIC SLUDGE DIGESTION



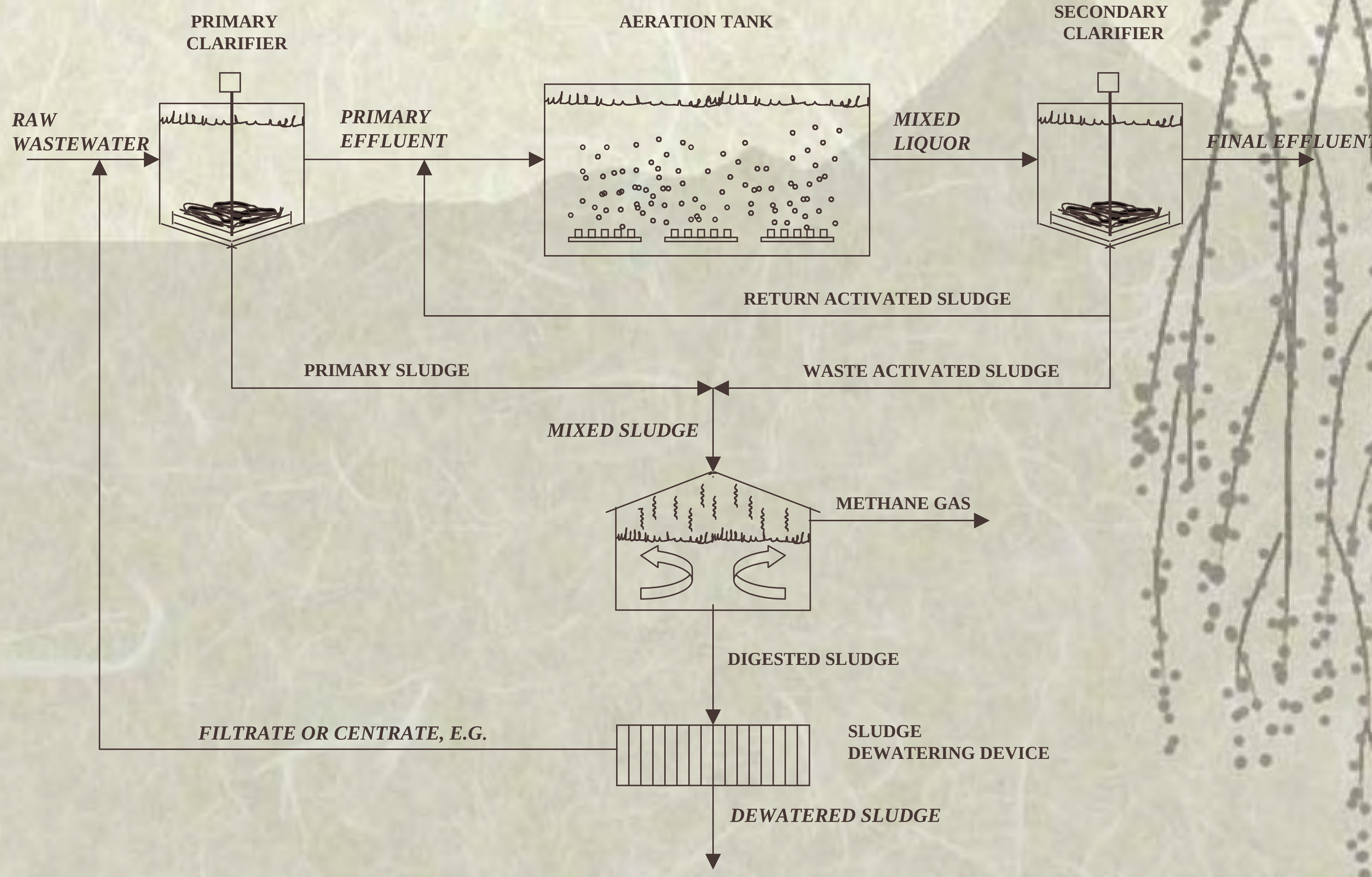
EDC PILOT PLANT FLOWSHEET WITH ANAEROBIC SLUDGE DIGESTION



EDC PILOT PLANT FLOWSHEET WITH AEROBIC SLUDGE DIGESTION



EDC PILOT PLANT FLOWSHEET WITH ANAEROBIC SLUDGE DIGESTION



EDC Pilot Plant

Aerobic Tanks

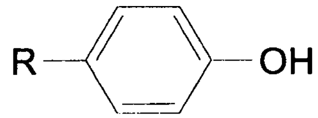
Secondary Clarifier

Anaerobic Digester

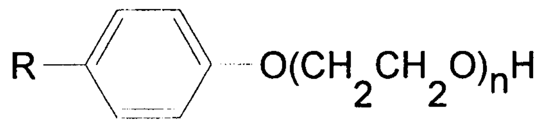
Make recommendations to Improve Treatment of Estrogenic EDCs

- Analyze results of pilot scale systems and modified pilot systems
- Compare results to field results (POTWs)
- Report the generated data and make final recommendations for improved Estrogenic EDC removal. Published as either a journal Article or EPA report

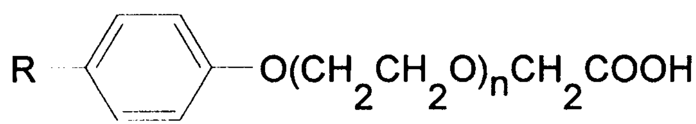
Alkylphenols and Ethoxylates



Alkylphenol



Alkylphenol polyethoxylate



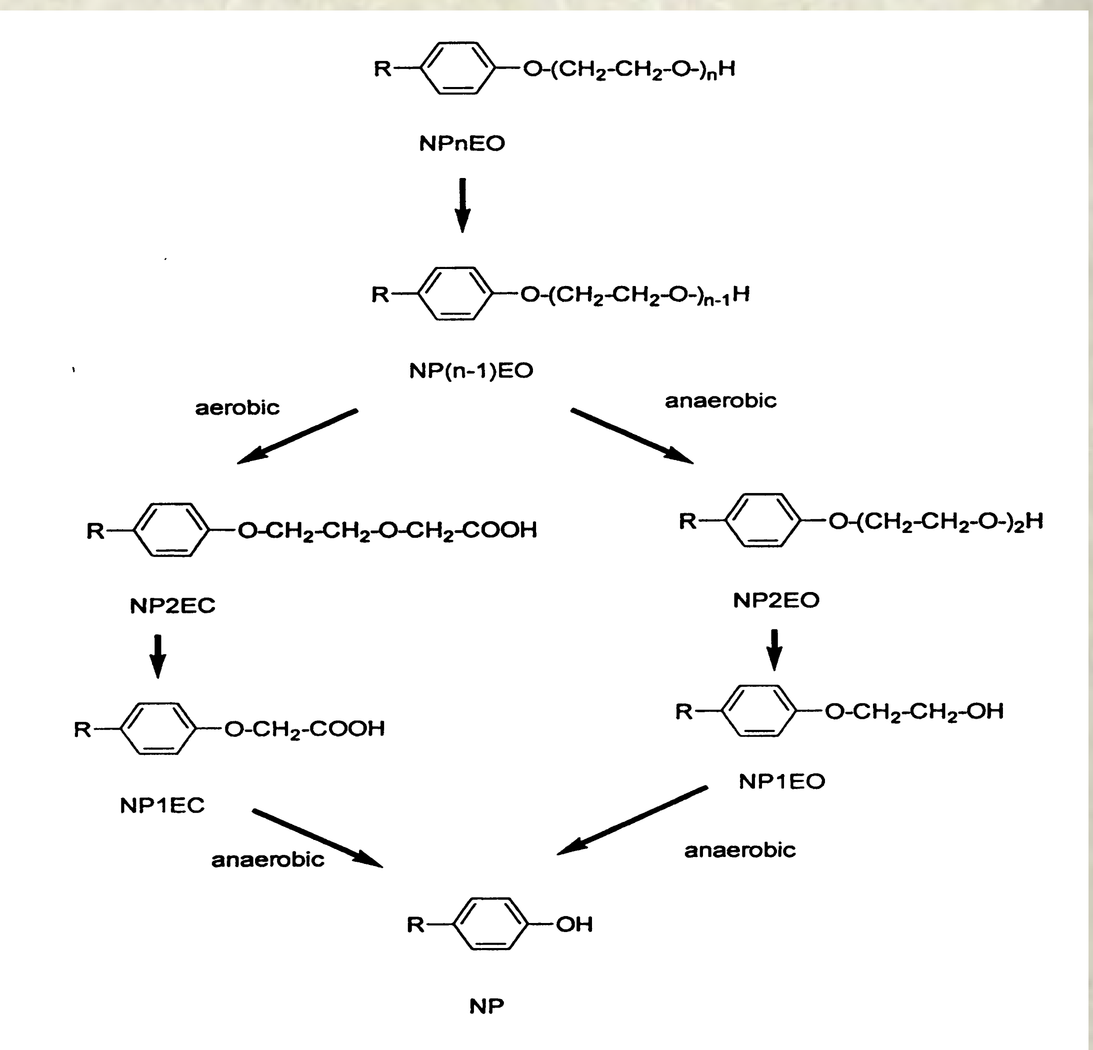
Alkylphenol polyethoxycarboxylate

$R = C_8H_{17}$ (octyl)

$= C_9H_{19}$ (nonyl)

R is usually branched

Metabolic fate of Alkylphenolics



Risk Management Research: Improving Environmental Decisions

Workshop on Effective Risk Management of Endocrine Disrupting Chemicals

Cincinnati, Ohio
January 29, 2002

Hugh W. McKinnon, M.D., M.P.H.
Associate Director for Health
National Risk Management Research Laboratory
U.S. Environmental Protection Agency

US EPA Office of Research and Development

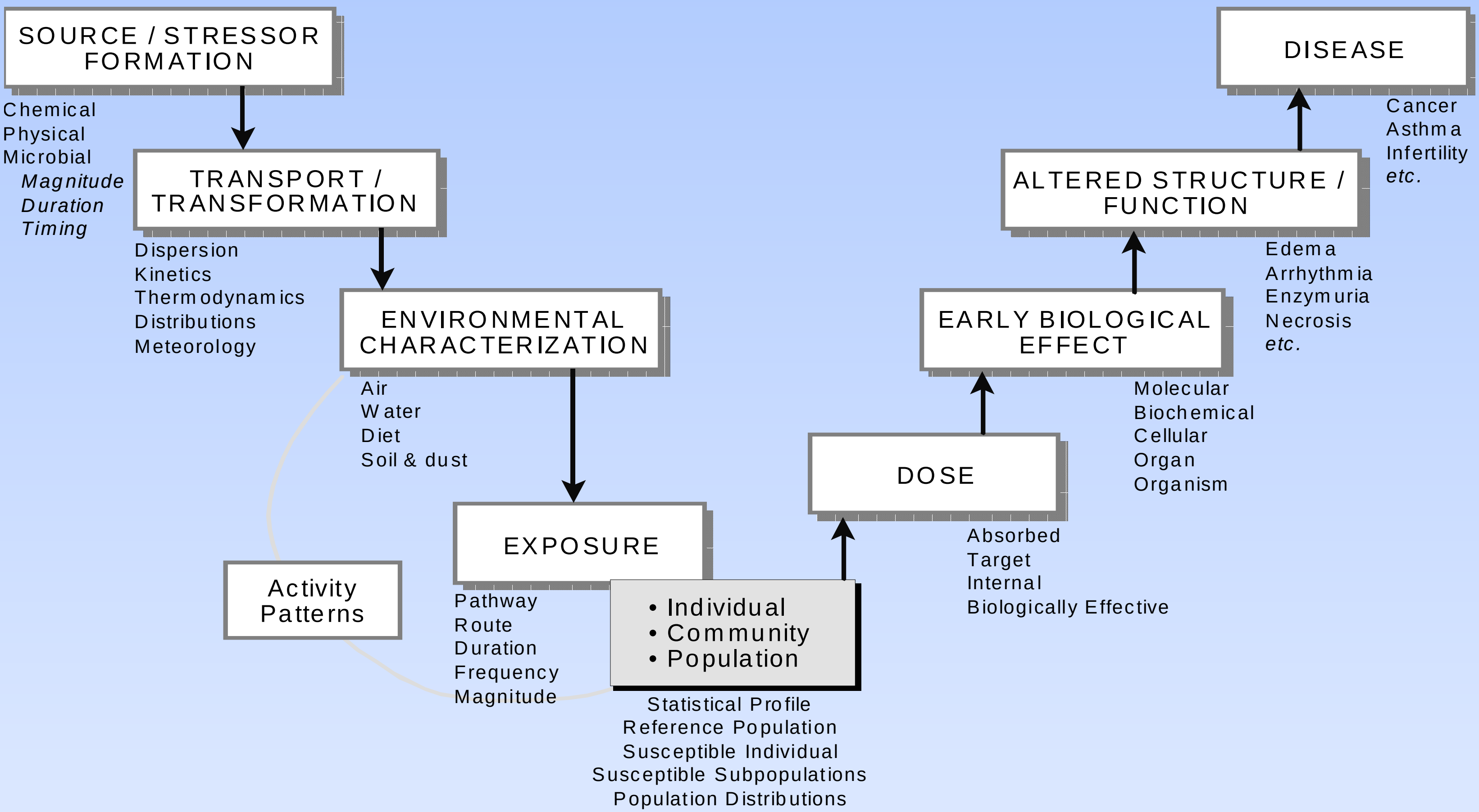
Risk Assessment/Risk Management Paradigm

Historical Perspective Focused on Uncertainty

“The dominant analytic difficulty [in decision-making based on risk assessments] is pervasive uncertainty...There is often great uncertainty in estimates of the types, probability and magnitude of health effects associated with a chemical agent, of the economic impacts of a proposed regulatory action, and of the extent of current and possible human exposures.”

“Risk Assessment in the Federal Government” (National Research Council, 1983)

Source-Exposure-Dose-Effect Continuum

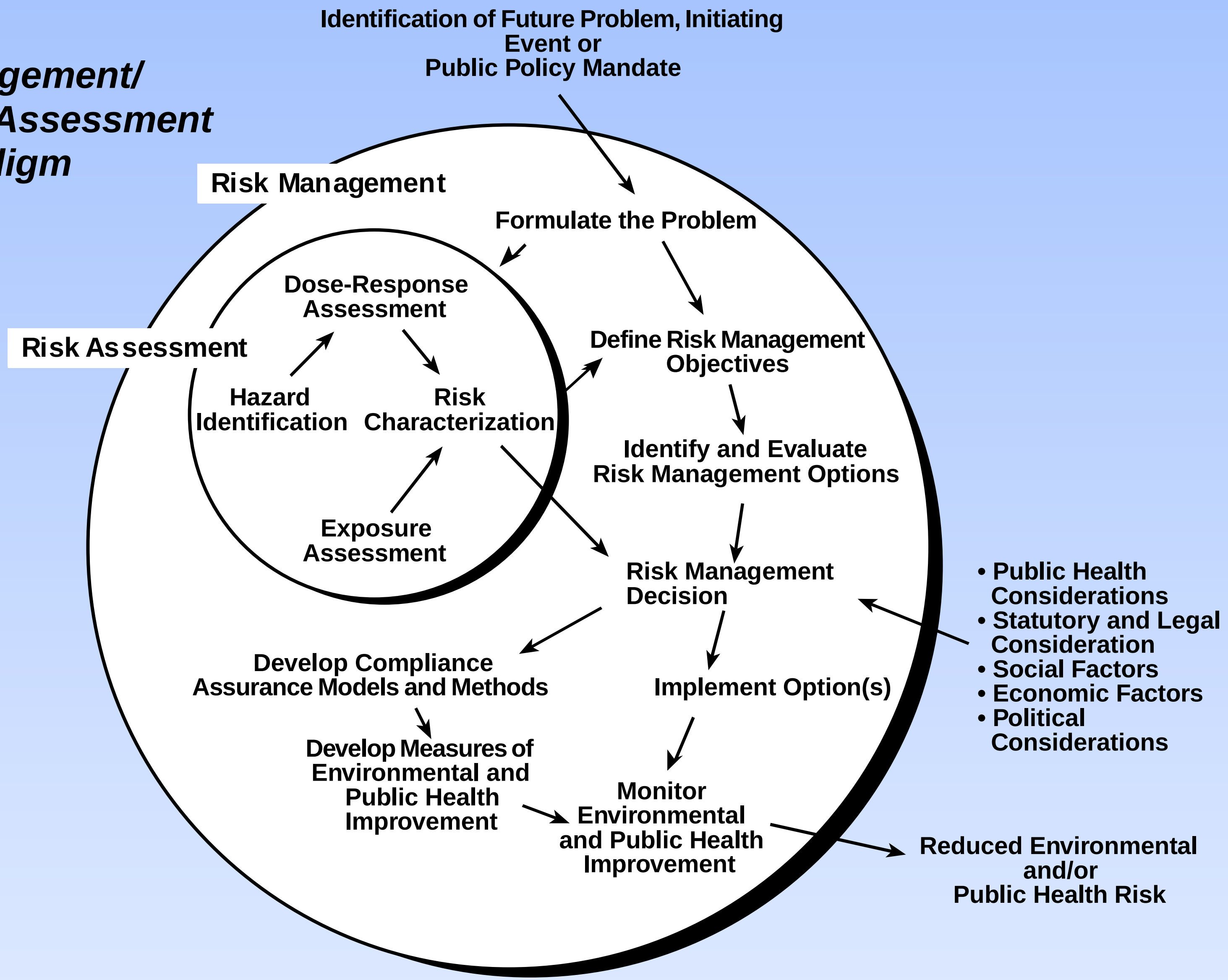


Recent Emphasis Focuses on the Use of Mechanistic Data

"The quality of risk analysis will improve as the quality of input improves. As we learn more about biology, chemistry, physics, and demography, we can make progressively better assessments of the risks involved. Risk assessment evolves continually, with re-evaluation as new models and data become available."

"Science and Judgment in Risk Assessment" (National Research Council, 1994)

Risk Management/ Risk Assessment Paradigm



ENVIRONMENTAL RISK

Fields of Analysis

Risk Assessment

- Nature of effects
- Potency of agent
- Exposure
- Population at risk
  - ➔ *Average risk*
  - ➔ *High-end risk*
  - ➔ *Sensitive groups*
- Uncertainties of science
- Uncertainties of analysis

Identify

Describe

Measure

Risk Management

- Social importance of risk
- De minimis or acceptable risk
- Reduce/not reduce risk
- Stringency of reduction
- Economics
- Priority of concern
- Legislative mandates
- Legal issues
- Risk perception

Evaluate

Decide

Implement

OBSERVATIONS ON RISK-BASED DECISION MAKING IN EPA

•Risk assessment and risk management within EPA are rarely if ever separated by sharp lines. Rather, decision-making is an interative process that considers available data and information on both the risks in question and the remedies available to mitigate the risks.

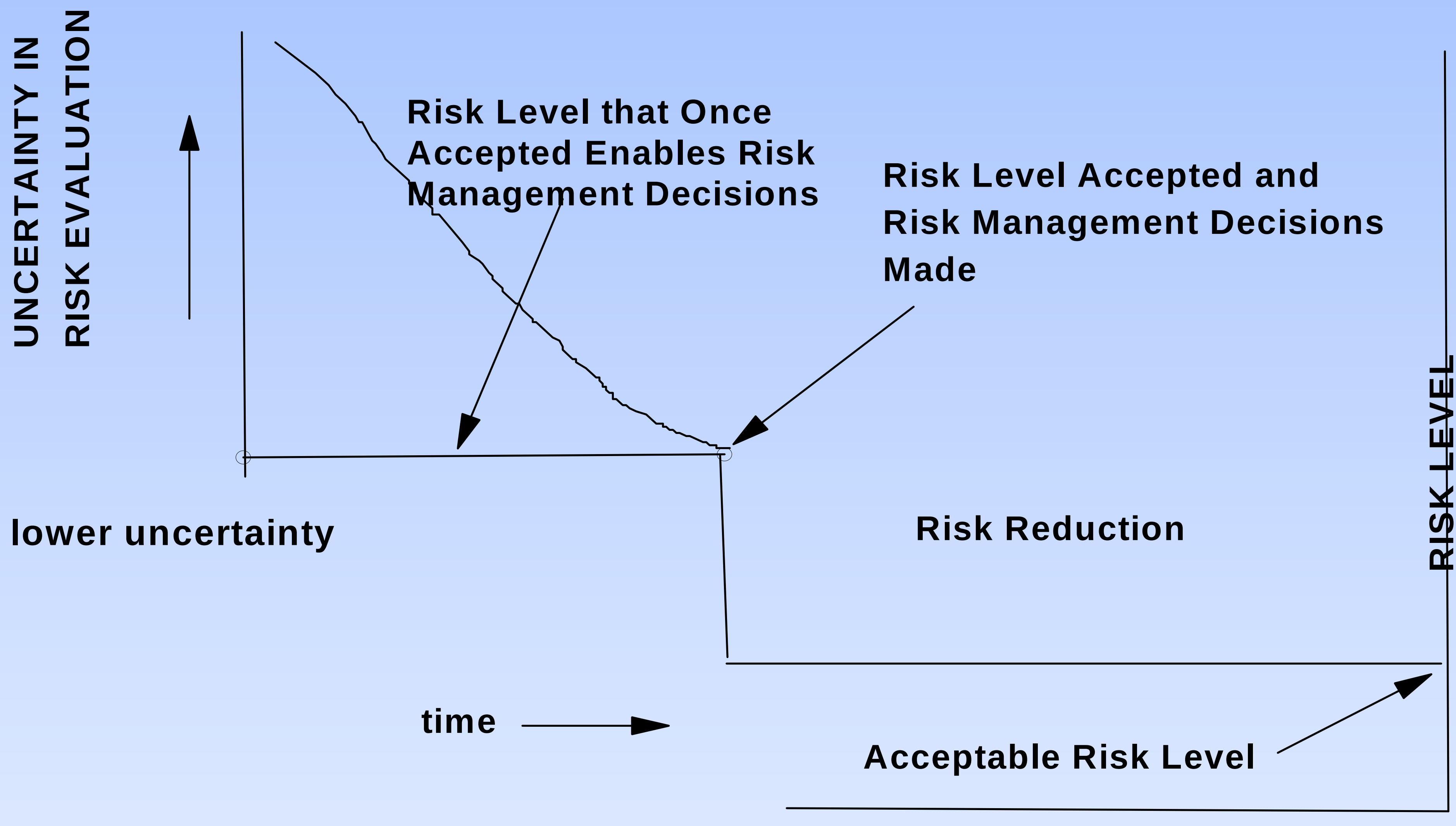
•The uncertainties, costs, commercial viability, and feasibilities attendant to available remedies influence the rigor demanded from all assessments required to define the problem and assess the risks.

OBSERVATIONS ON RISK-BASED DECISION MAKING IN EPA (cont.)

- Where legal or political mandates are clear and precedents are unambiguous, risk management decisions tolerate high degrees of uncertainty in both risk assessments and risk management remedies, especially when costs are modest.

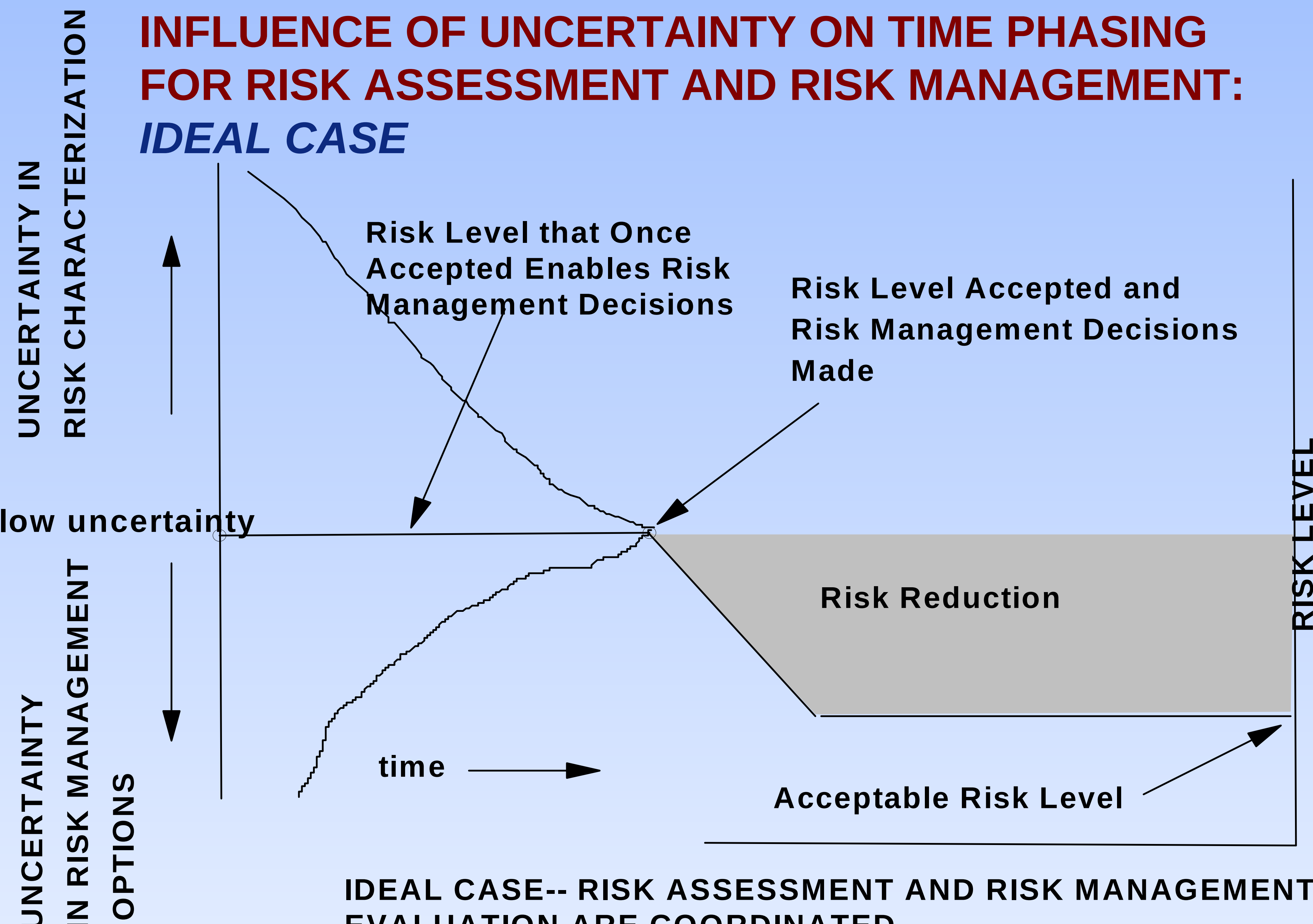
- Similarly, as the Agency moves toward advocacy (e.g., incentives, community-based programs, emission-trading, National action Plans) as a means to foster better decision-making and when costs of decisions are high, uncertainties in both risk assessment and risk management must be reduced.

INFLUENCE OF SCIENTIFIC UNCERTAINTY ON TIME PHASING OF RISK MANAGEMENT DECISIONS



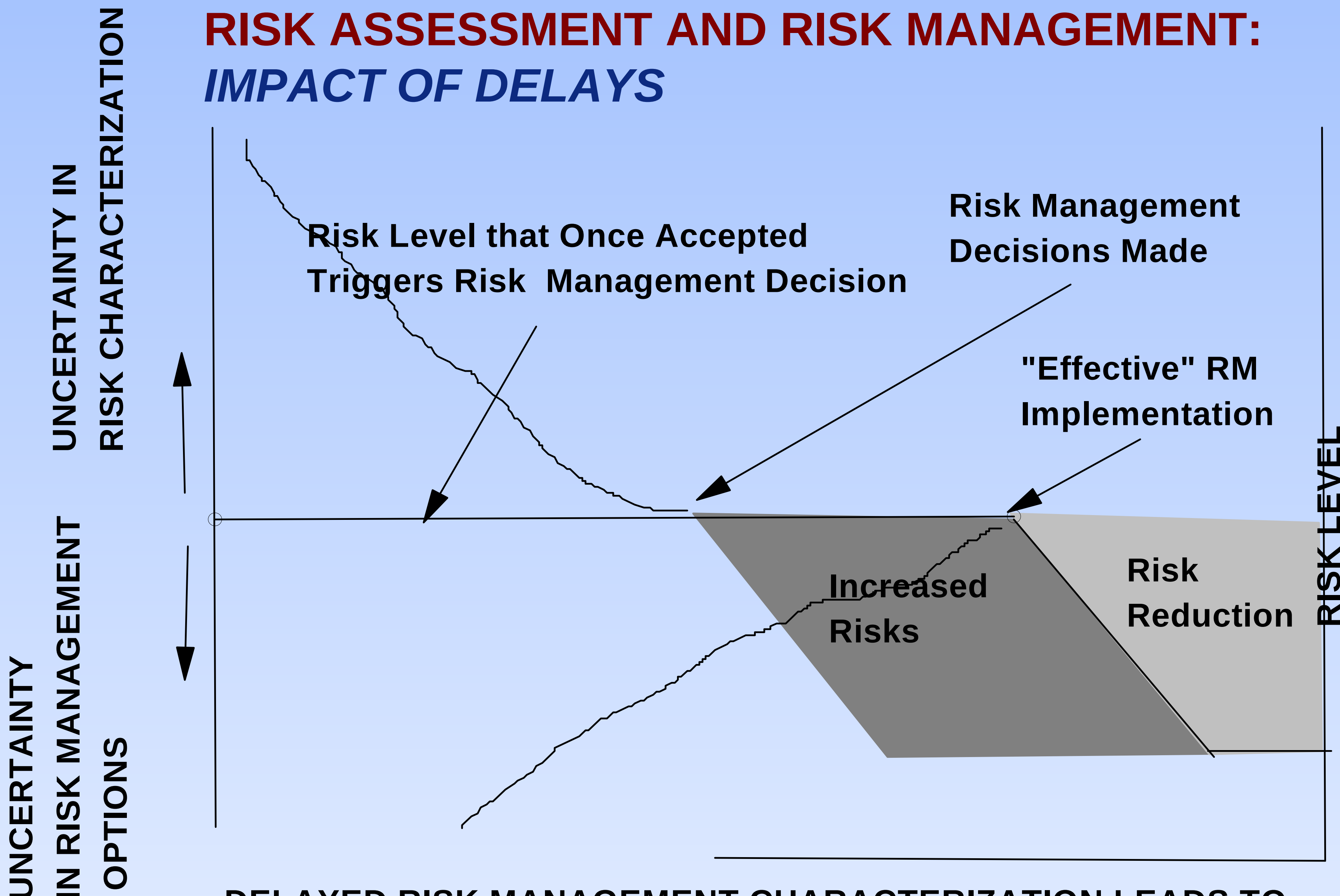
WHAT ABOUT UNCERTAINTIES IN RISK MANAGEMENT?

INFLUENCE OF UNCERTAINTY ON TIME PHASING FOR RISK ASSESSMENT AND RISK MANAGEMENT: *IDEAL CASE*



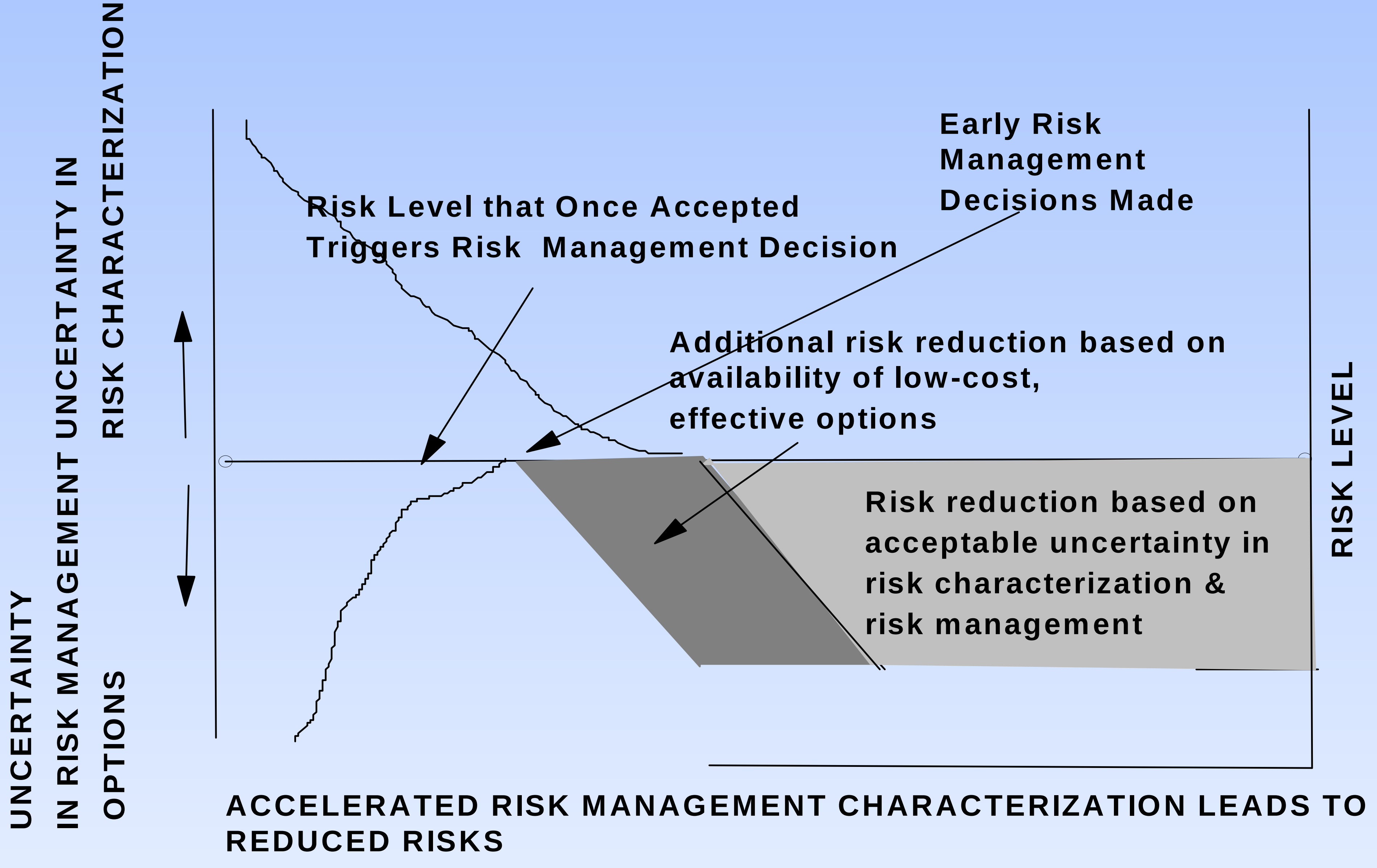
IDEAL CASE-- RISK ASSESSMENT AND RISK MANAGEMENT EVALUATION ARE COORDINATED

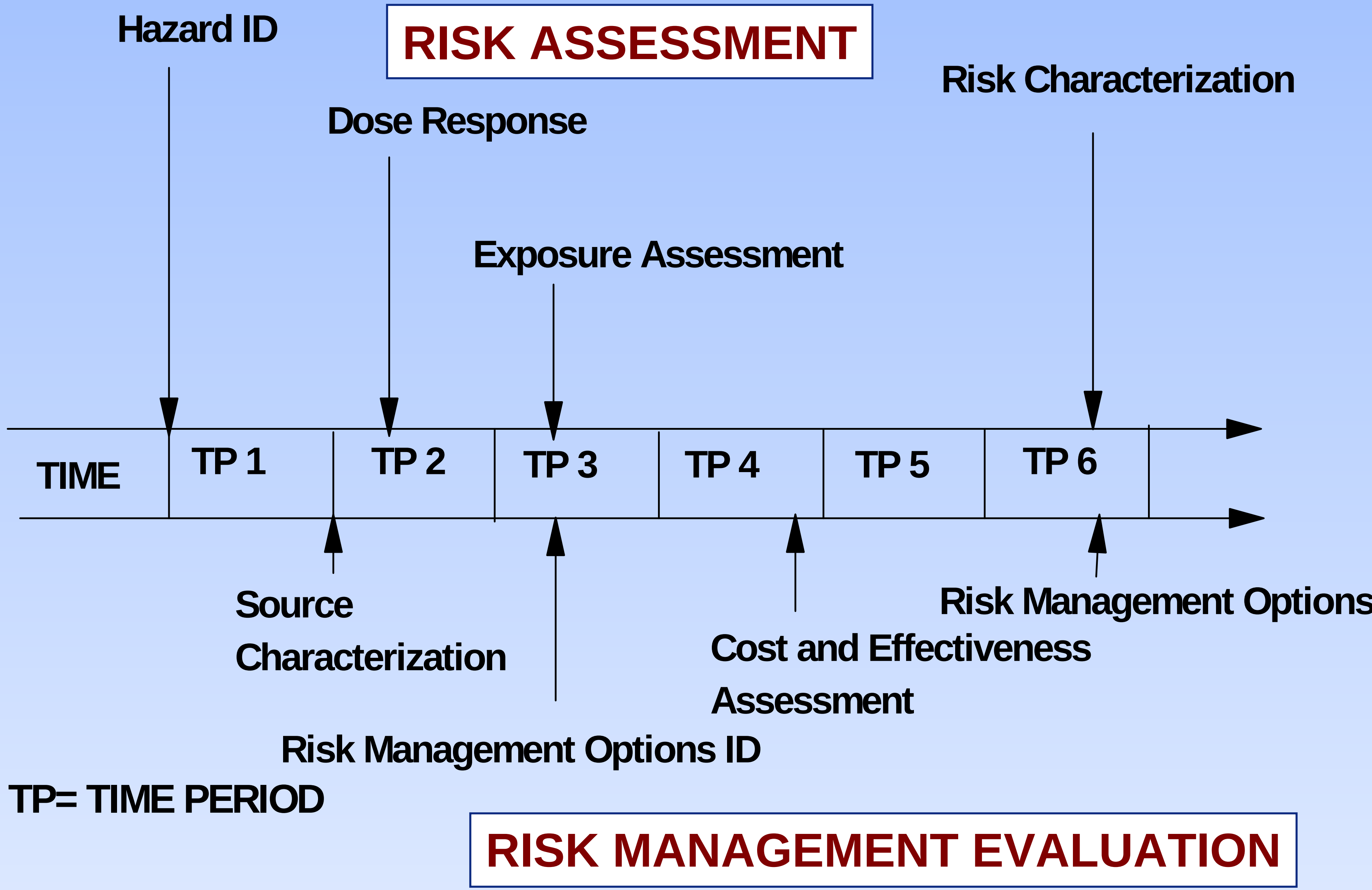
INFLUENCE OF SCIENTIFIC UNCERTAINTY ON RISK ASSESSMENT AND RISK MANAGEMENT: *IMPACT OF DELAYS*



DELAYED RISK MANAGEMENT CHARACTERIZATION LEADS TO INCREASED RISKS

INFLUENCE OF SCIENTIFIC UNCERTAINTY ON RISK ASSESSMENT AND RISK MANAGEMENT: IMPACT OF ACCELERATED RISK MANAGEMENT CHARACTERIZATION





TIME PHASING FOR RISK ASSESSMENT AND RISK MANAGEMENT CHARACTERIZATION TO ACHIEVE OPTIMAL RISK REDUCTION

RISK MANAGEMENT EVALUATION

DEFINITION:

An analysis of:

- sources of potential, perceived, or actual risk,
- RM options for preventing or reducing risk, (primarily technical, e.g., control, P2)

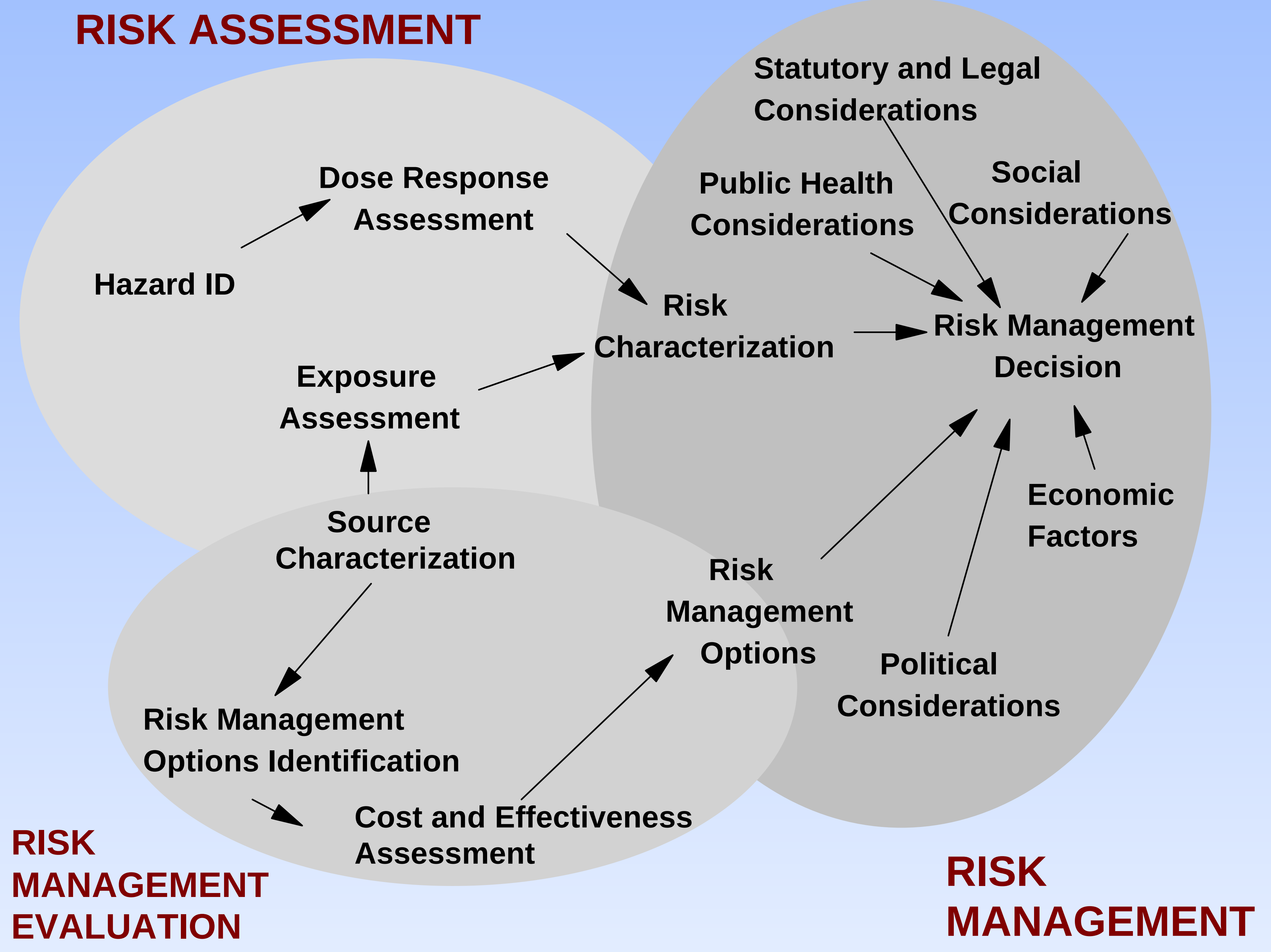
and

- availability, costs and effectiveness of the identified RM options.

RISK MANAGEMENT EVALUATION

GOAL OF RISK MANAGEMENT EVALUATION RESEARCH:

Reduce uncertainty in Risk Management for EPA and other public and private sector organizations and provide cost-effective RM options (both technical and non-technical) for preventing, reducing, or adapting to current and emerging high risk problems.



RISK ASSESSMENT
Statutory and Legal Considerations
Dose Response Assessment
Public Health Considerations
Social Considerations
Hazard ID
Risk Characterization
Risk Management Decision
Exposure Assessment
Source Characterization
Economic Factors
Risk Management Options
Political Considerations
Risk Management Options Identification
Cost and Effectiveness Assessment
RISK MANAGEMENT EVALUATION
RISK MANAGEMENT

RISK MANAGEMENT EVALUATION

Source Characterization

- identification--who, what, where
- source strengths--how much, what form
- timing--changes over time
- emission factors--estimation

RISK MANAGEMENT OPTIONS

Risk Management Options Identification

- pollution prevention
- source control technologies
- management practices
- remediation
- restoration
- adaptation

Effectiveness and Cost Assessment

- verification status--commercially available?
- known effectiveness--data
- estimated effectiveness--engineering judgment
- reliability estimates--failure modes & rates
- capital costs
- O&M costs

PILOT RISK MANAGEMENT EVALUATIONS UNDERWAY IN ORD

- Endocrine Disruptors
- Arsenic In Drinking Water
- CAFOs
- Particulate Matter ($PM_{2.5}$)

Excerpt from "Risk Management Research: Improving Environmental Decisions"
Hugh McKinnon, NRMRL
EDC Workshop January 29, 2002

In 1995, the Office of Research and Development in EPA, re-organized into our current configuration, creating three national laboratories and two national centers. At that time, we became the National Risk Management Research Laboratory, and remembering that our predecessors had been primarily engineering and technology development laboratories, we decided to take a look at the risk management side of the process. We felt the risk assessment side had received a fair amount of scrutiny and study, but the risk management side had not. So we decided to try to draw a better depiction of the risk management process, and among the senior management in ORD collectively came up with this diagram. I don't know who to credit, but someone alertly observed that the risk assessment process is at the core of this, fits into the whole matter of making risk management decisions, and came up with this diagram that looks like a cell, with the risk assessment as the nucleus. Out here, in very simplistic form, are laid out some of the major steps in making a risk management decision, implementing that decision, and looking at what the consequences are.

There is a great variety of risk assessment, risk management, and other considerations, that come into play here. So as we consider decision making, keep in mind that we are the research and development arm of EPA–the people who try to provide scientific and technical support to the rest of the Agency. We are not the risk managers, we're not those who, in the Agency, are setting the regulatory standards, nor those at the state and local levels who are actually having to make decisions and implement them.

But, in looking at the process, we came up with some observations. One is that risk assessment/risk management are rarely sharply separated. Decision making tends to be iterative and it considers all the data available. Secondly, there are uncertainties, as well as costs and other considerations, that play into risk management and risk assessment, and those have not really been looked at and defined so well in decision making. Third, we observed that, where legal or political mandates are clear and where we have unambiguous precedents, risk management decisions will tolerate a great deal of uncertainty in both the risk assessment and remedies, especially if the costs are not high. If the costs are high however, or if the precedents are not so clear, then the uncertainty question comes into play and people begin to demand a great deal of reduction in uncertainty.

Finally, as our Agency moves toward less of a command-and-control approach and more toward what we've called an advocacy approach, things like incentives, community based programs, consideration of trading programs, action plans that are so broadly defined at the national level but left for local implementation and decision, and when the costs of decisions are high, then uncertainties in both risk assessment and risk management need to be reduced. That, in a nutshell, is what we've tried to do since the formation of the Risk Management Research Lab. We're continuing to try to do that, continuing to try to bring increased definition of our risk management understanding and programs that broaden the scope of discipline that those might include, and that will continue.

Along the way, Lee Mulkey came up with the idea of something we're calling a risk management evaluation. The risk management evaluation is a summary of what we know about risk management options at the time a decision needs to be made. It would include an analysis of sources of risks, the risk management options that exist for preventing or reducing those risks, and the availability, cost, and effectiveness of those options. Again it was not our intent to second guess or re-hash the complex risk assessments that often accompany Agency decisions. It was rather to look at the other side of the paradigm, if you will, at the risk management side. In order to do that, we might indeed have to provide a capsule summary of what we know about risks. So the goal of these evaluations and the research to support them is to reduce uncertainty in risk management for EPA and others, in order to provide them with cost effective risk management options. These might be technical or non-technical, for preventing, reducing or adapting to current and emerging high-risk problems. So we came up with this diagram to encapsulate what we were saying about the risk management evaluation. You will recognize this is the risk assessment side of the paradigm, and this is the risk management side and we've added a third oval to summarize what's included in a risk management evaluation. Hopefully, the risk management evaluation complements the risk assessment and plays into effective risk management decision making.

Development of Chemical Methods to Characterize Exposure to EDCs in the Neuse River Basin

M. Medina-Vera, S. Harper, L. Wright, E. Coppedge, S. Lumpkin

US EPA/ORD/NERL/HEASD

G. Ferrell

USGS/Raleigh

United States Environmental Protection Agency

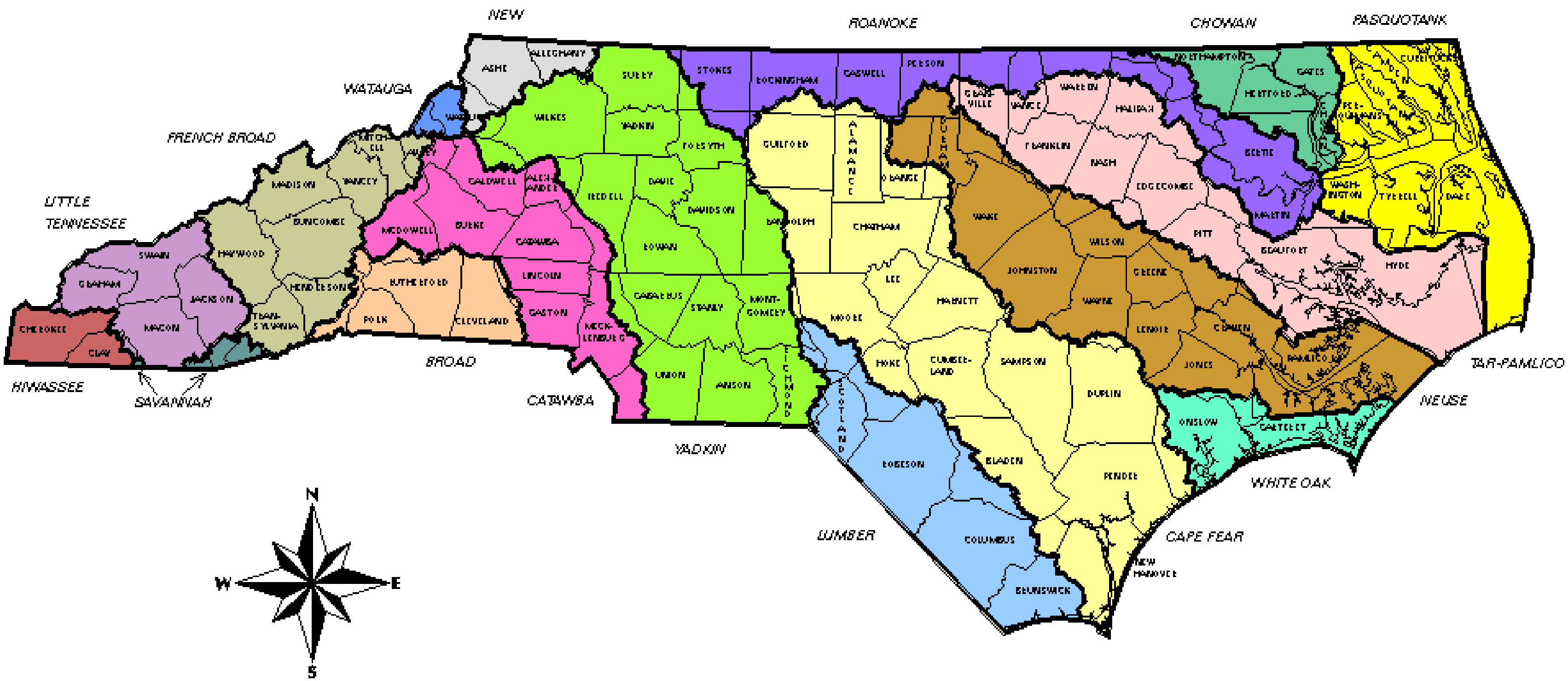
US EPA Office of Research & Development

USGS science for a changing world

EPA Near Laboratory Ecological Research Areas (NLERA)

- Cincinnati, OH- Little Miami River Basin
- Las Vegas, NV-Colorado River Basin
- Athens, GA- Savannah River Basin
- Research Triangle Park, NC- Neuse River Basin

NORTH CAROLINA RIVER BASINS



SCALE 1:5,000,000

0 100 200 MILES

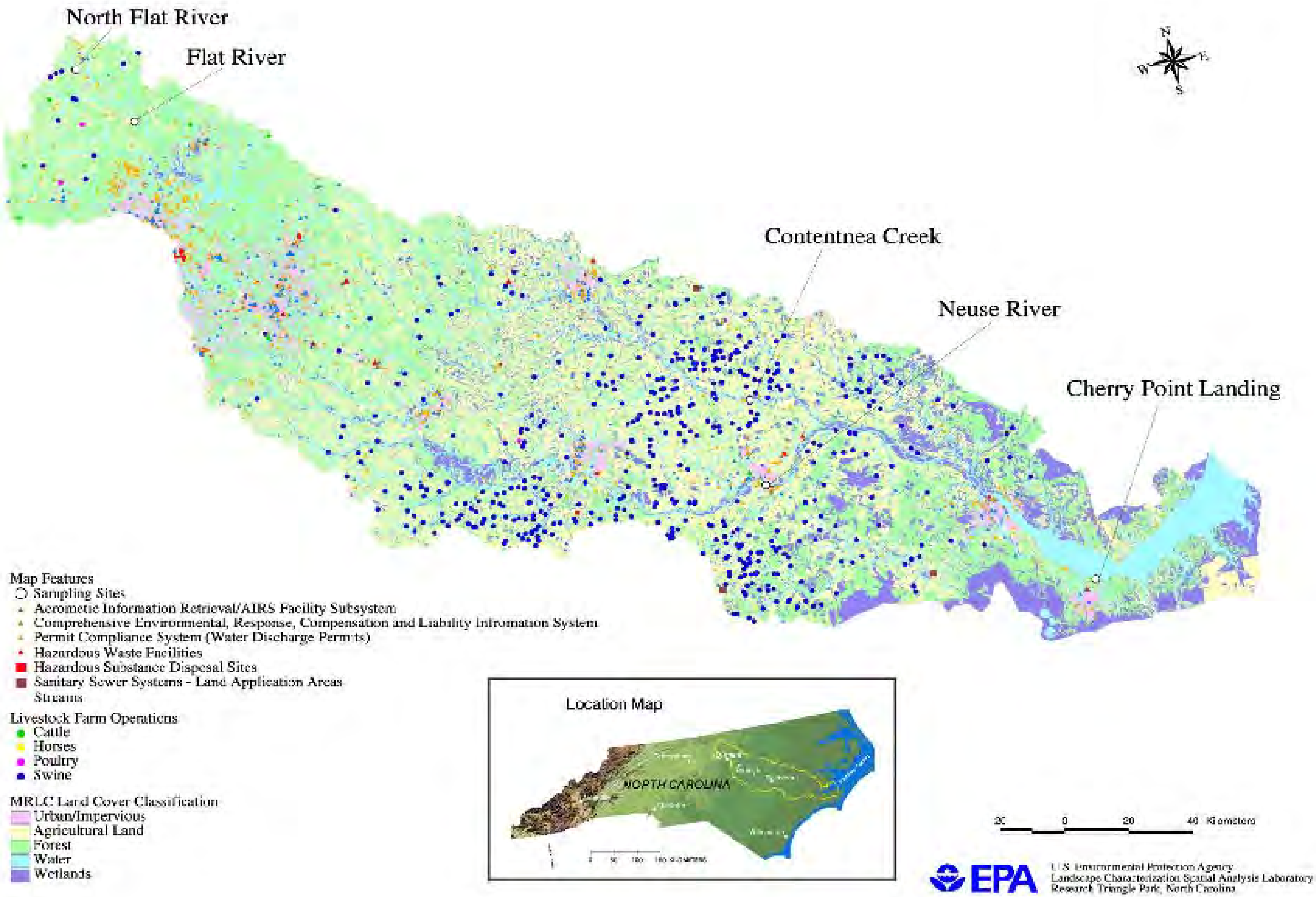
Map Prepared by the N.C. Geographic Information & Analysis * 115 Hillsborough Street * Raleigh, NC 27603 * (919)733-2090

EPA United States Environmental Protection Agency

ORD US EPA Office of Research & Development

USGS science for a changing world

Potential Sources of Toxic Elements in the Neuse River Basin



Methods Development

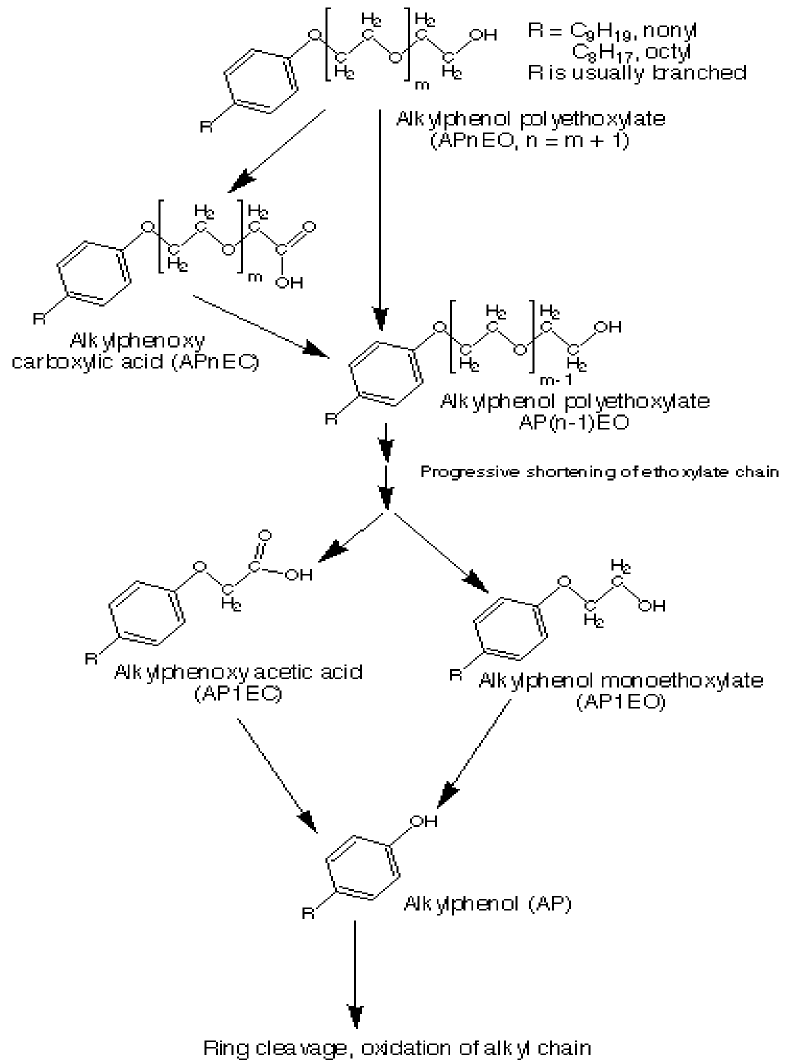
- Organic compounds
  - Alkylphenol Polyethoxylates (APEOs)
  - Antibiotics
  - Pesticides
- Inorganic compounds
  - Toxic Elements
    - Focus: As, Cd, Cu, Mn, Pb, Sn

Alkylphenol Polyethoxylate Research

- Why are they important?
  - Breakdown products are considered more toxic than parent compounds.
  - Persistent in rivers, sediments, and groundwater.
  - Bioaccumulated by fish and birds.
  - Considered as EDC mimickers.

- Sources/Uses (Examples)
  - Manufacturing: pulp and paper, textile, plastic & elastomer, leather.
  - Household, industrial, and institutional cleaning products, spermicides in contraceptive gels, jellies, creams.
  - Agriculture.

Degradation Mechanism of APEOs



Organic Compounds Sampling Methods

- Water: Amber collection bottles dipped at least 5” deep upstream side of the boat.
- Sediment: Grab samples (1/25 m^2) stainless steel coated with Kynar.
- Fish: USGS method adaptation of Report 93-104.

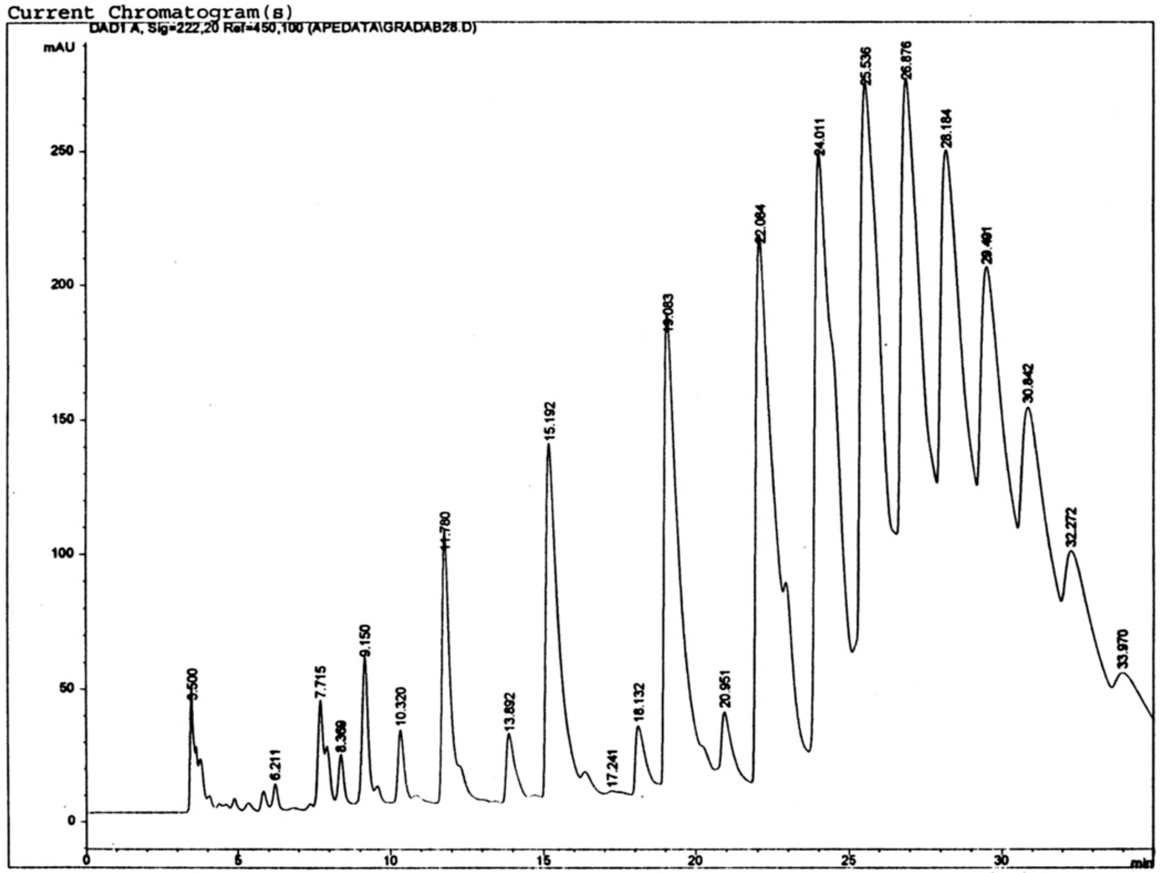
Analytical Methods

- Isolation/Extraction Methods
  - Solid Phase Extraction (SPE): Water, Sewage.
  - Accelerated Solvent Extraction (ASE): Sediment

- Chromatographic Methods
    - HPLC Reverse Phase: ODS Hypersyl, 100% H_2O/0.005 $KH_2(PO_4)$ to 100% CH_3CN, 30 minutes, 0.250 mL/min flowrate. Reverse Phase: 100% Methanol Isocratic, 35 minutes.
    - HPLC Normal Phase: NH_2 column, gradient 10% Isopropanol, 90% Hexane to 100% Isopropanol, 0% Hexane, 35 minutes.

Normal Phase Chromatography

Print of window 38: Current Chromatogram(s)



Instrument 1 3/21/2001 10:03:43 AM LWright

Page 1 of 1

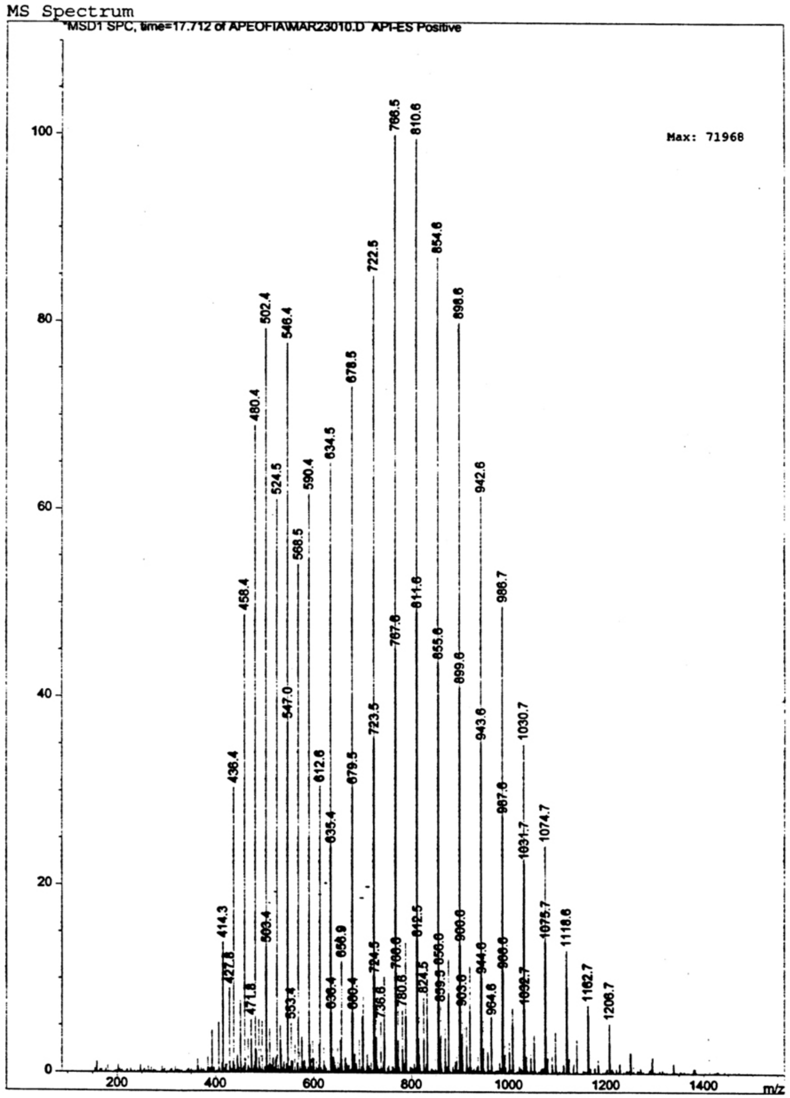
EPA United States Environmental Protection Agency

US EPA Office of Research & Development

USGS science for a changing world

Mass Spectrum

Print of window 80: MS Spectrum



lcms 3/23/01 10:26:02 AM ferrell

Page 1 of 1

United States Environmental Protection Agency

US EPA Office of Research & Development

USGS science for a changing world

Toxic Elements Research

- Why are they important?
  - Persistent, bioaccumulates, toxic.
  - Impacts on reproductive, immune, neuro-behavioral and endocrine system functions in developing and mature organisms.

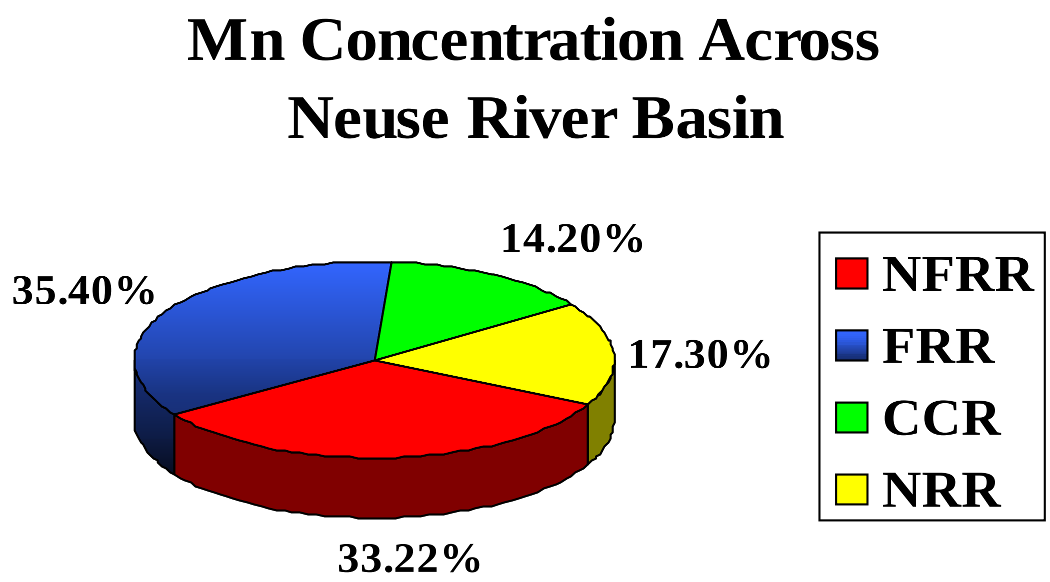
- Sources/Uses (Examples)
  - Used as growth promoters for chickens.
  - Herbicides, wood preservatives, semiconductor manufacturing, petroleum refining, mining and smelting operations.
  - Medical and municipal waste incinerators, utility boilers, paints, fish.

Toxic Elements Methods

- Example: Surface Water Sampling
  - EPA Method 1669, “Sampling Ambient Water for Trace Metals at EPA Quality Criteria Levels”.
- Analytical
  - Confirmation: Absorption Spectroscopy.
  - Modified EPA Method 1639, “Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption”.
  - Modified EPA Method 200.9 “Determination of Trace Elements by Stabilized Temperature Graphite Furnace Atomic Absorption”.

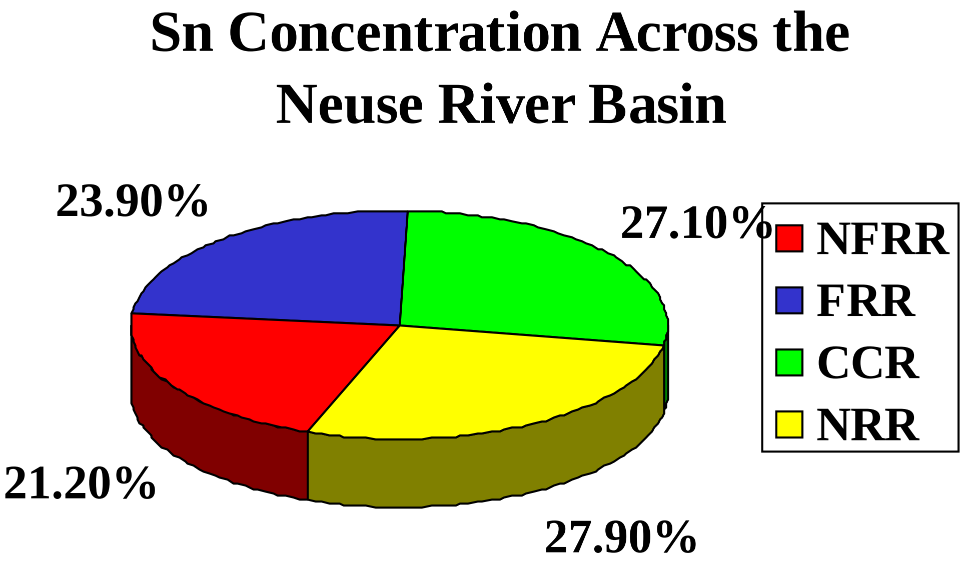
Toxic Elements Results-1

- NFRR: North Flat River Reach
- FRR: Flat River Reach
- CCR: Contentnea Creek Reach
- NRR: Neuse River Reach



Toxic Elements Results-2

- NFRR: North Flat River Reach
- FRR: Flat River Reach
- CCR: Contentnea Creek Reach
- NRR: Neuse River Reach



Future Directions

- Continue developmental work on field and analytical methods.
- Provide environmental baselines for targeted compounds/elements at established sites in the Neuse River Basin.
- Continue research with the Regional Offices on APEOs.

Acknowledgments

- EPA/ORD/NERL/ESD: Landscape Characterization Branch, RTP, NC: Neuse River Landscape data & coverages.

- EPA/ORD/NERL/HEASD/IO-RTP, NC : Peter Knudsen-graphics support.

Excerpt from “Welcome from the National Risk Management Research Laboratory”
Lee Mulkey, NRMRL
EDC Workshop January 29, 2002

As I look at this program, it strikes me that there are three things that are somewhat different about this topic and about the way we organized the work. First of all, as a way of introducing the idea, the budget category in our organization that we typically identified EDCs with is something we call Emerging Risks. That’s an important idea because, as you know, EPA typically responds and reacts to issues that come up. We looked at a river that burned in Cleveland years ago, Superfund was spun up after incidents like Love Canal and Times Beach, and a whole new industry on bioremediation was generated from an oil tanker rupture in Alaska. Here is a chance where EPA has had the foresight to consider what may be a real risk that requires either new policy development or new initiatives in implementing or calibrating our regulatory programs dealing with EDCs.

There are two other things that come to mind that I want to share with you before we get down to the real work. One is that one surely needs to worry about the footprint of EDCs. Now let me explain a bit what I mean by this. Ecologists typically talk about ecological footprints of human activities and the matrix they use. As you may know, these are the per-capita appropriations and areal extent of the natural environment that we humans appropriate for those goods and services we get. One of the important services that we appropriate from the environment is its assimilative capacity for our waste and our residuals. Now one surely must wonder–what’s the footprint of EDCs? We may not think about EDCs as a waste, but you’re going to hear in this program about the sources and the sinks, and although we’ll continue to depend upon the environment to assimilate EDC residuals, the extent of our use and the areal extent of releases are still quite large. So surely, one must appropriately wonder about the risks and try to get a handle on them.

Now the third thing that this idea of an Emerging Risk gives us the opportunity to do is to engage in what we might phrase ‘integrated science.’ We are welcoming you on behalf of the Risk Management Research Laboratory, but this topic reaches across all of the research elements within EPA. And, in a parallel fashion, we are trying to develop our knowledge base by asking: what are the risks of EDCs, what are the effects, what’s the extent of the exposure, what are the probabilities of the scenarios and ways and places where intervention might be appropriate, what are the appropriate options for managing the risks once they’re identified and where and how much, and at what cost, and toward what benefits? This is a good model, I think, for how we ought to think about integrating our science and our research in order to inform the policy making steps that may be required at EPA and the federal government, at the state agencies and, through interaction with our colleagues, at the international community.

Risk Management of Endocrine Disrupting Chemicals (EDCs) in Drinking Water

Frederick W. Pontius, P.E.
Gary Amy, Ph.D.

Objective

- Present a water utility perspective on EDC risk management
  - Are current regulations adequate?
  - Should we be concerned about unregulated EDCs/pharmaceuticals?
  - What can water utilities do to manage risk?
  - What are the critical research questions?

Acknowledgements

- Gretchen Watson, Univ. of Colorado
  - SEM images
- Prof. Scott Summers, Univ. of Colorado
- AWWA Research Foundation
  - Workshop held April 19-21, 2000

Are Current Regulations Adequate?

- The Drinking Water Context
- Existing and Future Regulations
- SDWA Authority

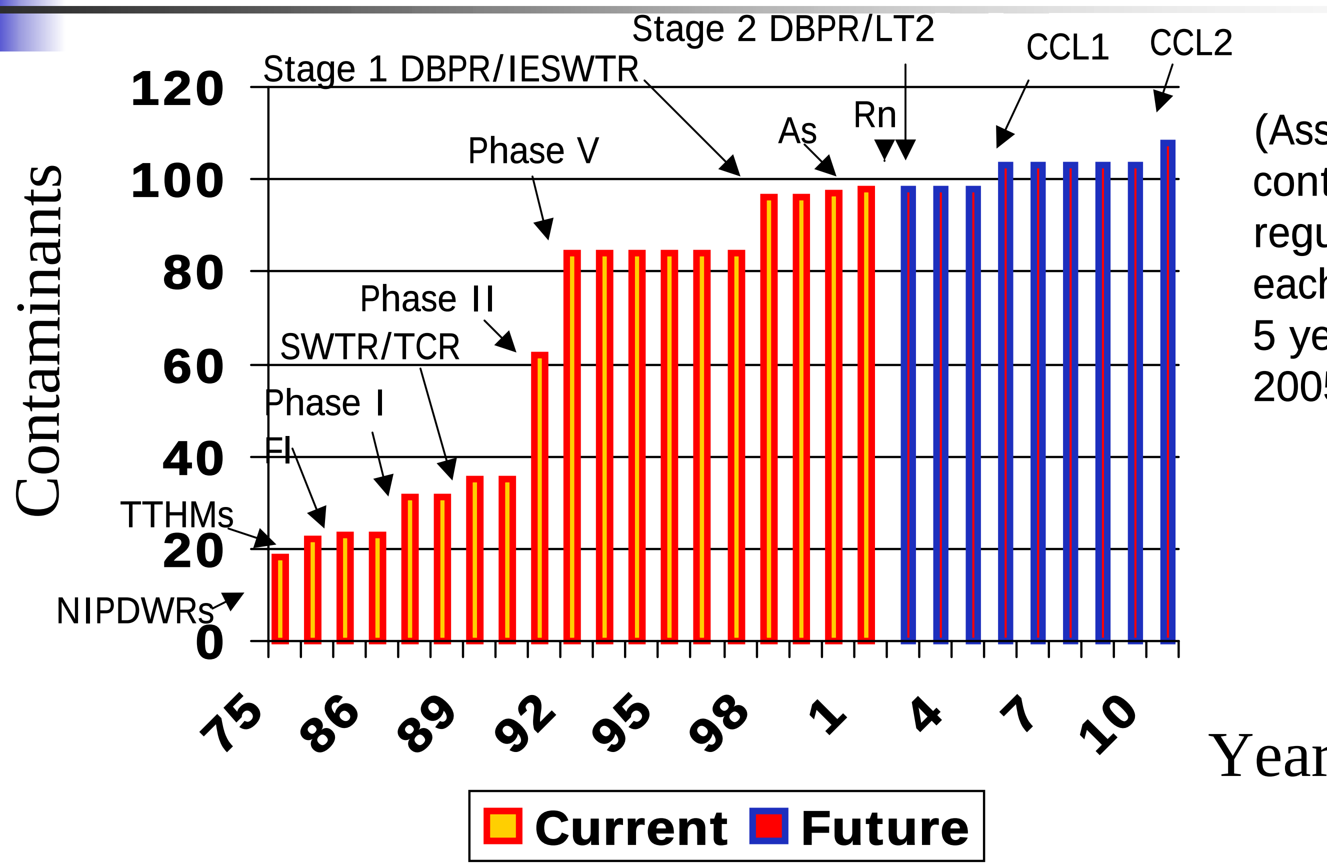
The Drinking Water Context

- 'Endocrine disruption' ➔ an effect...
- Disruption of the endocrine system is just one of many health effects that could be associated with a drinking water contaminant
  - Inorganic; Organic (Natural; Synthetic)
  - Cancer; Noncancer

Drinking Water Context (Cont.)

- Drinking water quality regulated by USEPA under the Safe Drinking Water Act (SDWA)
- Bottled water quality regulated by the Food and Drug Administration (FDA)
- Other environmental exposures regulated by other laws

Contaminants Regulated



(Assumes 5 contaminants regulated from each CCL every 5 years starting 2005.)

SDWA Authority

- USEPA regulates contaminants that...
  - Occur or are likely to occur in drinking water
  - May have adverse effects on human health
  - Have a meaningful opportunity for risk reduction
- Specific contaminants regulated
  - MCLG → MCL; BAT; Benefits/Costs; Risks

Should we be Concerned About Unregulated EDCs/Pharmaceuticals?

- Occurrence
- Health Risks
- Treatment Effectiveness

Occurrence and Health Risks

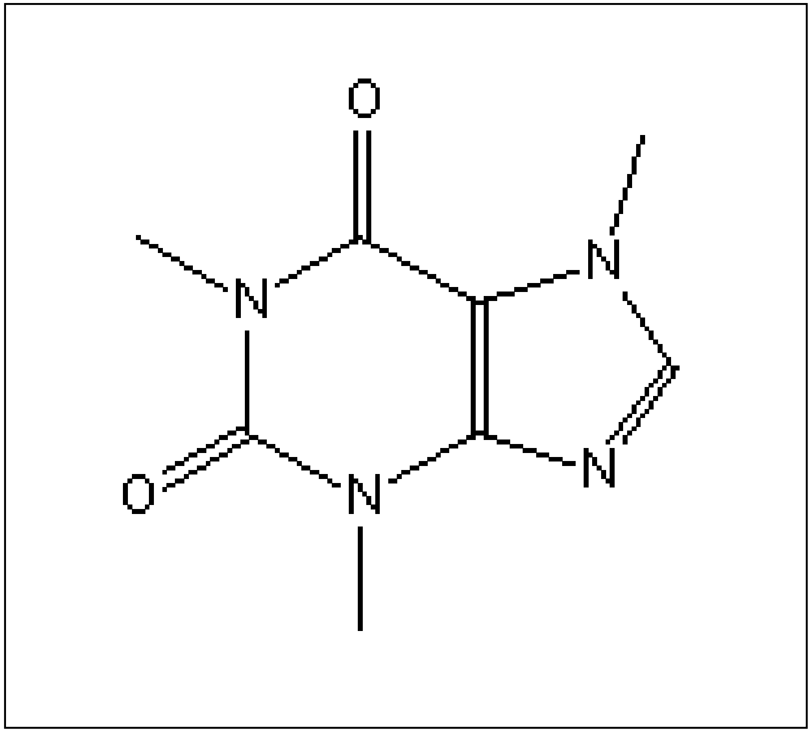
- Sometimes we find'em, most(?) of the time we don't
  - If detected, then we have evidence.
  - If not detected, then ?
  - Many types of chemicals, configurations
- Potential health effects may be significant, exposure and health risk uncertain

EDCs/Pharmaceuticals in Source Waters

- Inorganic (natural; discharges)
- Organic (natural; synthetic)
  - Specific contaminants
    - effluent dominated streams/lakes
  - Total Organic Carbon (TOC)
    - Dissolved organic matter (DOC)
    - Natural organic matter (NOM)
    - Effluent organic matter (EfOM)
    - Soluble Microbial Products (SMPs)
    - Algal organic matter (AlOM)

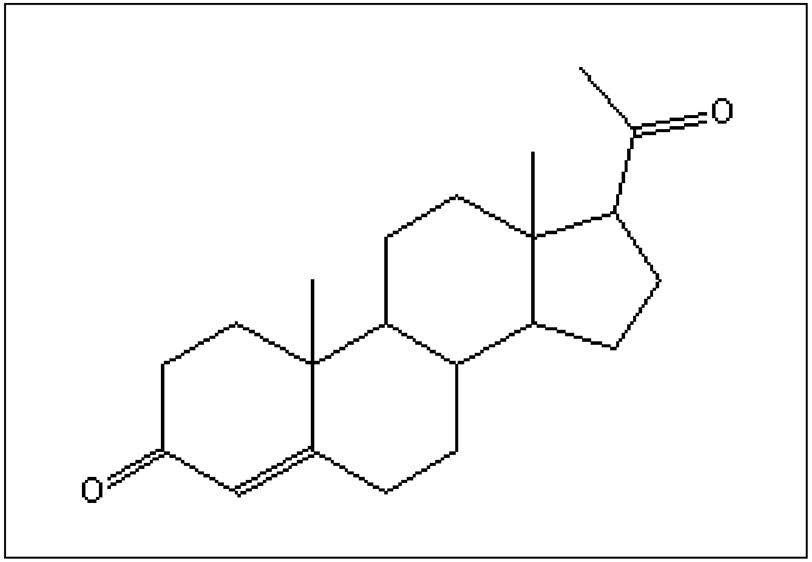
Caffeine

- $C_8H_{10}N_4O_2$
- MW = 194.19
- MP = 238 C
- C_w^{sat} = 21,600 mg/L
- Log K_{ow} = -0.07
- P^o = 15 mm Hg
- pKa = 10.4
- H = 1.9 x 10^{-19} atm-m^3/mole



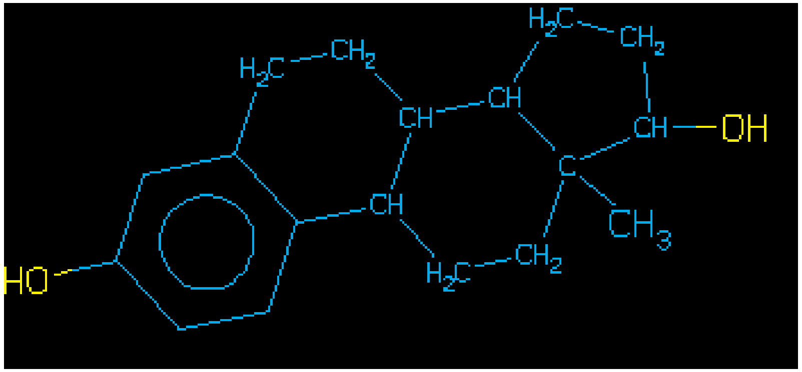
Progesterone

- $C_{21}H_{30}O_2$
- MW = 314.47
- MP = 121 C
- C_w^{sat} = 8.81 mg/L
- Log K_{ow} = 3.87
- P^o = 1.3 x 10^{-6} mm Hg
- H = 6.49 x 10^{-8} atm-m^3/mole



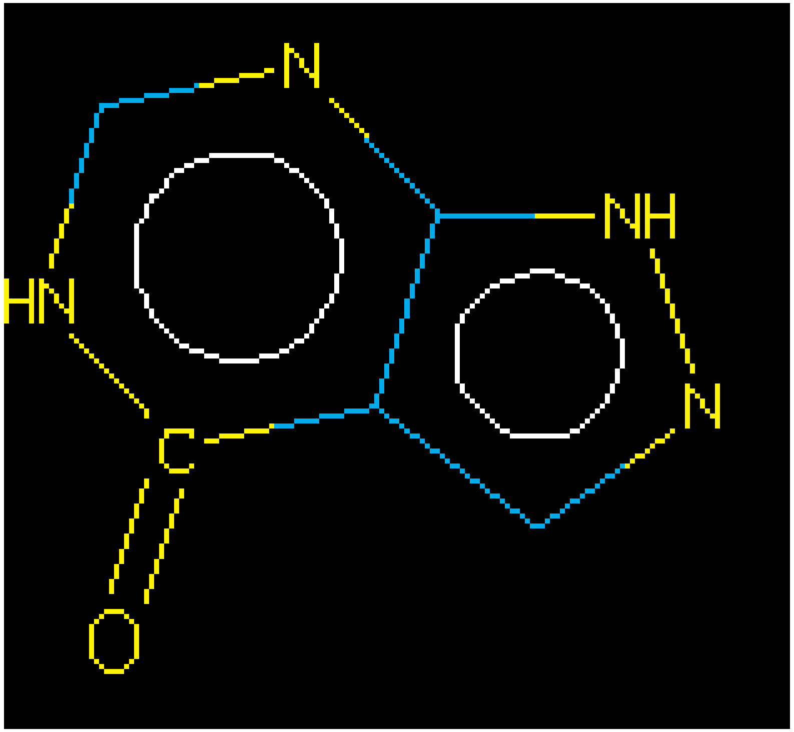
beta-Estradiol

- $C_{18}H_{24}O_2$
- MW = 272.39
- MP = 178.5 C
- C_w^{sat} = 3.6 mg/L
- Log K_{ow} = 4.01
- P^o = 1.26 x 10^{-8} mm Hg
- H = 3.64 x 10^{-11} atm-m^3/mole



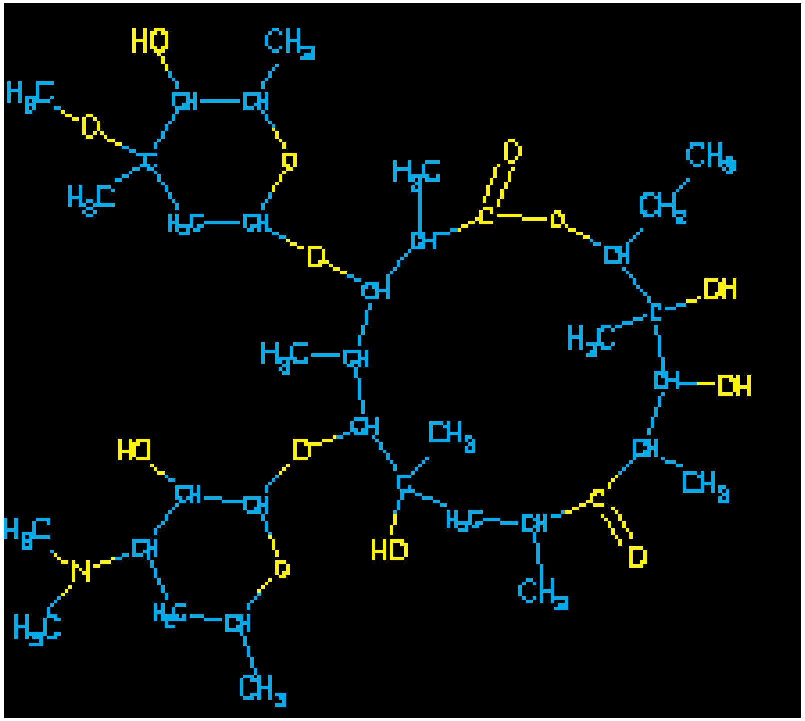
Allopurinol

- $C_5H_4N_4O$
- MW = 136.11
- MP = 350 C
- C_w^{sat} = 569 mg/L
- Log K_{ow} = -0.55
- P^o = 4.73 x 10^{-6} mm Hg
- H = 2.03 x 10^{-14} atm-m^3/mole

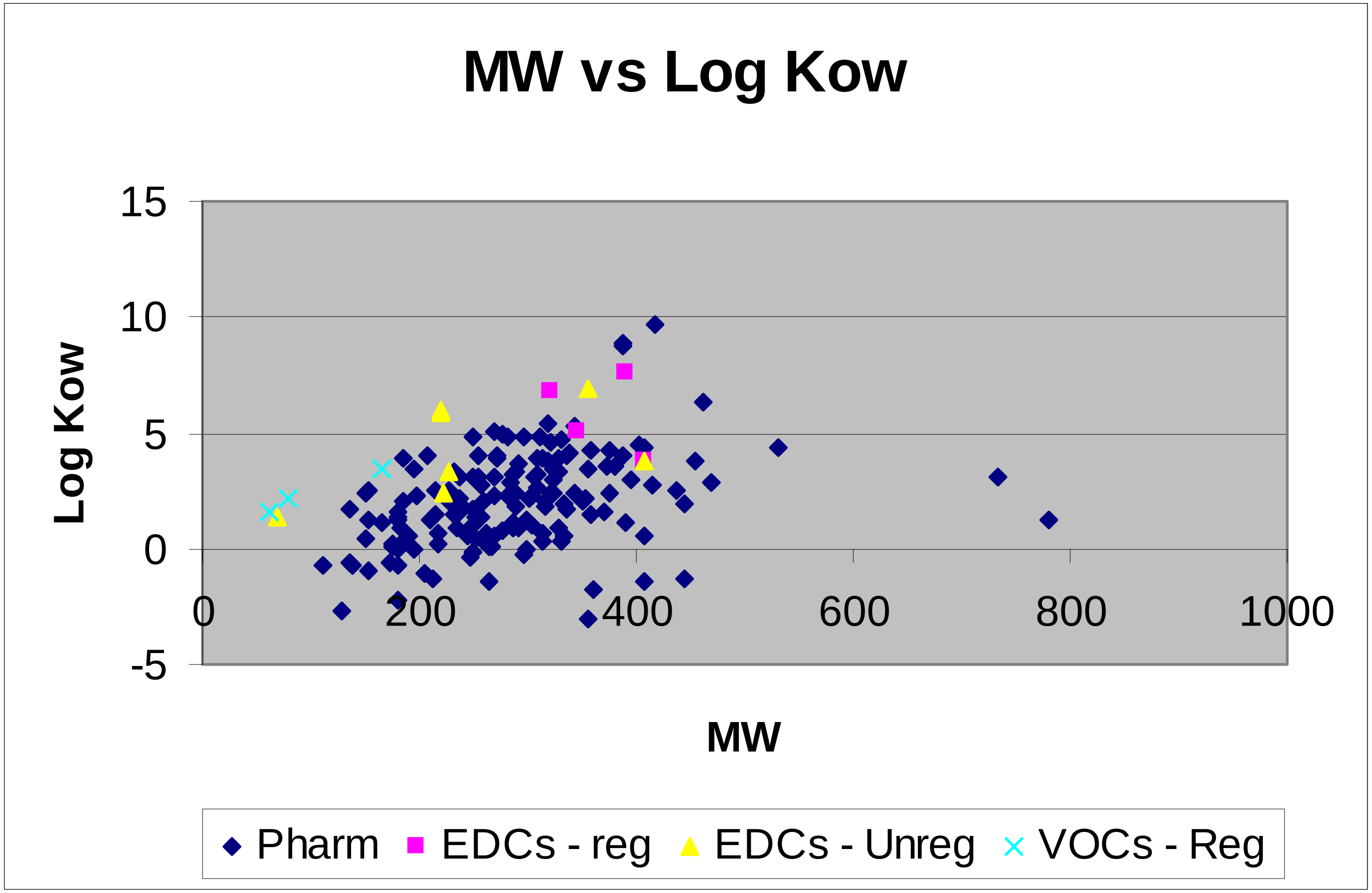


Erythromycin

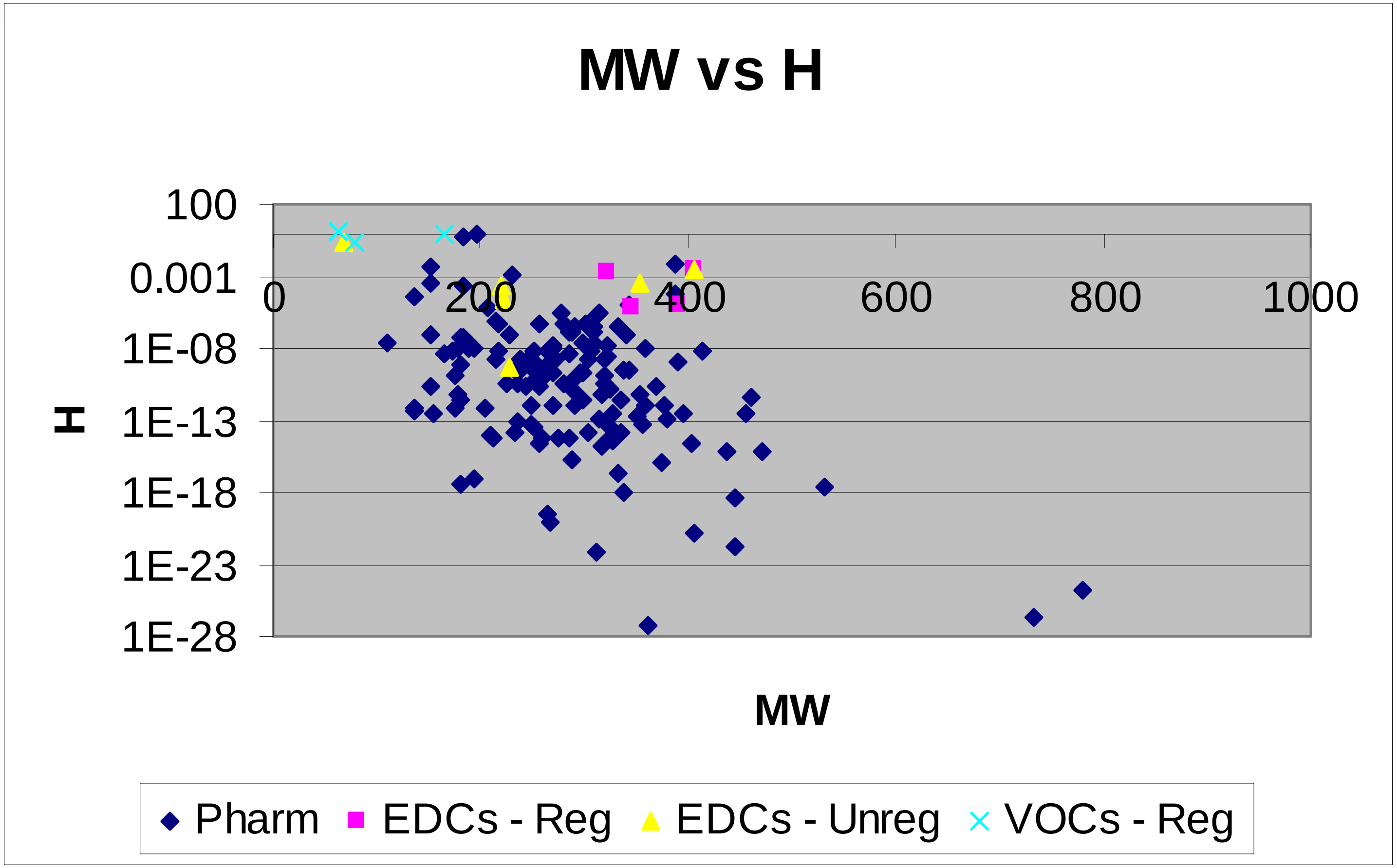
- $C_{37}H_{67}NO_{13}$
- MW = 733.95
- MP = 191 C
- C_w^{sat} = 1.4 mg/L
- Log K_{ow} = 3.06
- P^o = 2.28 x 10^{-27} mm Hg
- H = 5.42 x 10^{-29} atm-m^3/mole



EDC/Pharmaceuticals



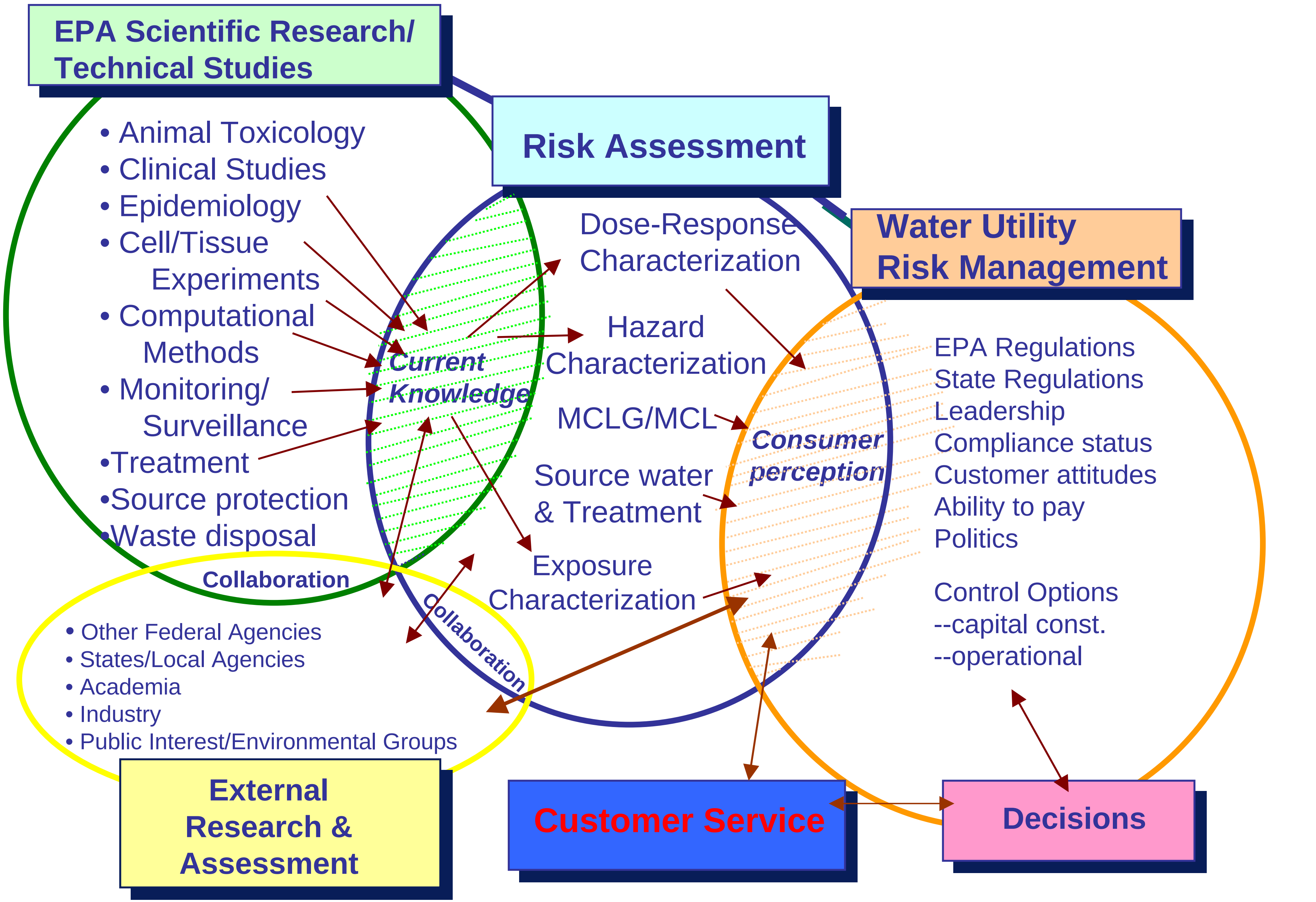
EDCs/Pharmaceuticals



What Can Water Utilities Do To Manage Risk?

- The water utility perspective
- Optimize using current regulatory control strategies
- Due diligence

Knowledge ⇄ Consumer ⇄ Utility



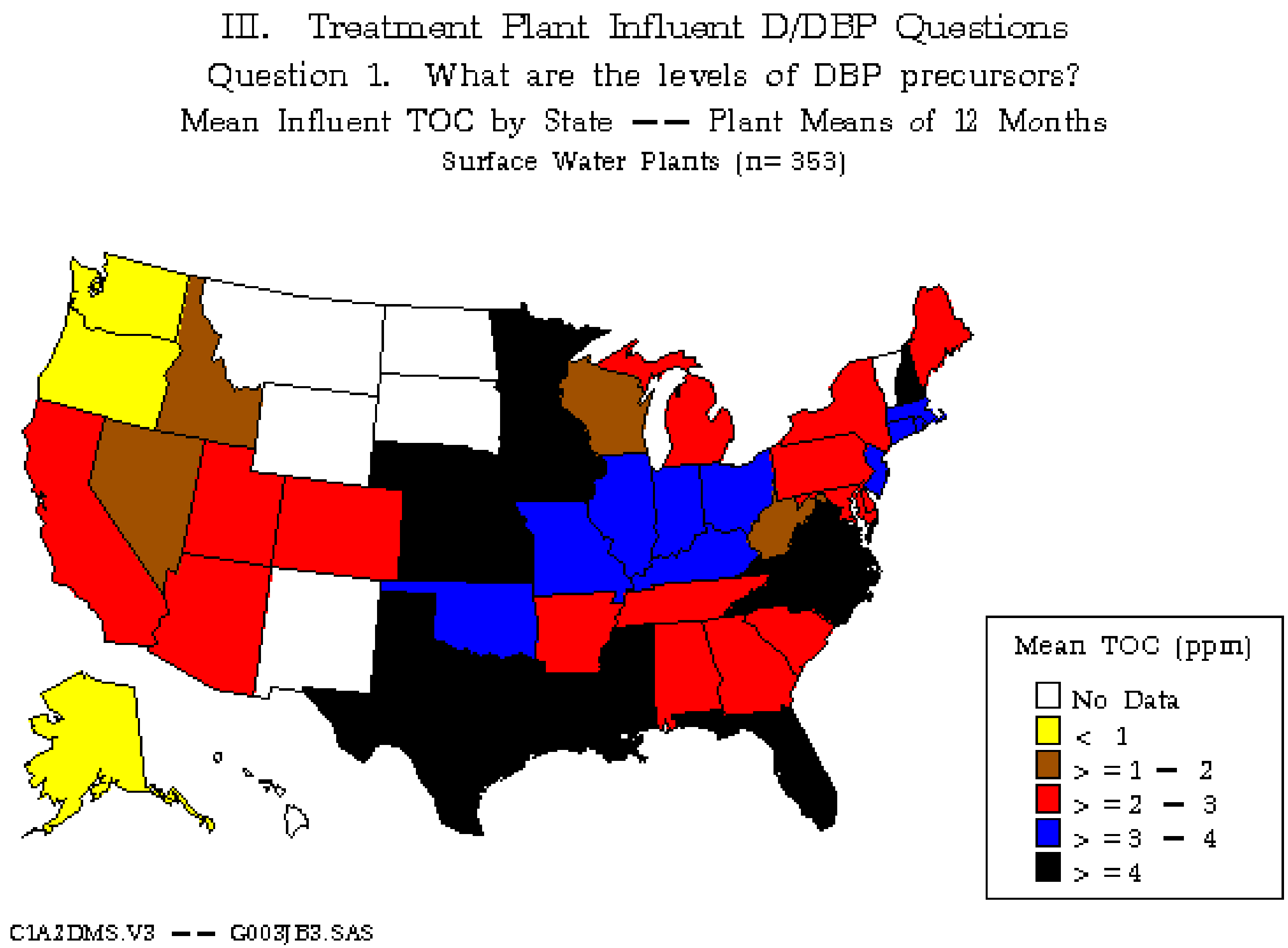
EDC/Pharmaceutical Control

J.M. Symons, 1994

Enhanced Coagulation (EC)

- SW - EC effective for NOM humic fraction
  - SUVA = UV254/DOC (L/mg-m)
  - Good indicator of water's humic content
  - Non-humics less amenable to enhance coagulation
  - SUVA </= 2.0 L/mg-m exempt from Stage 1 DBPR EC requirement

Surface Water TOC



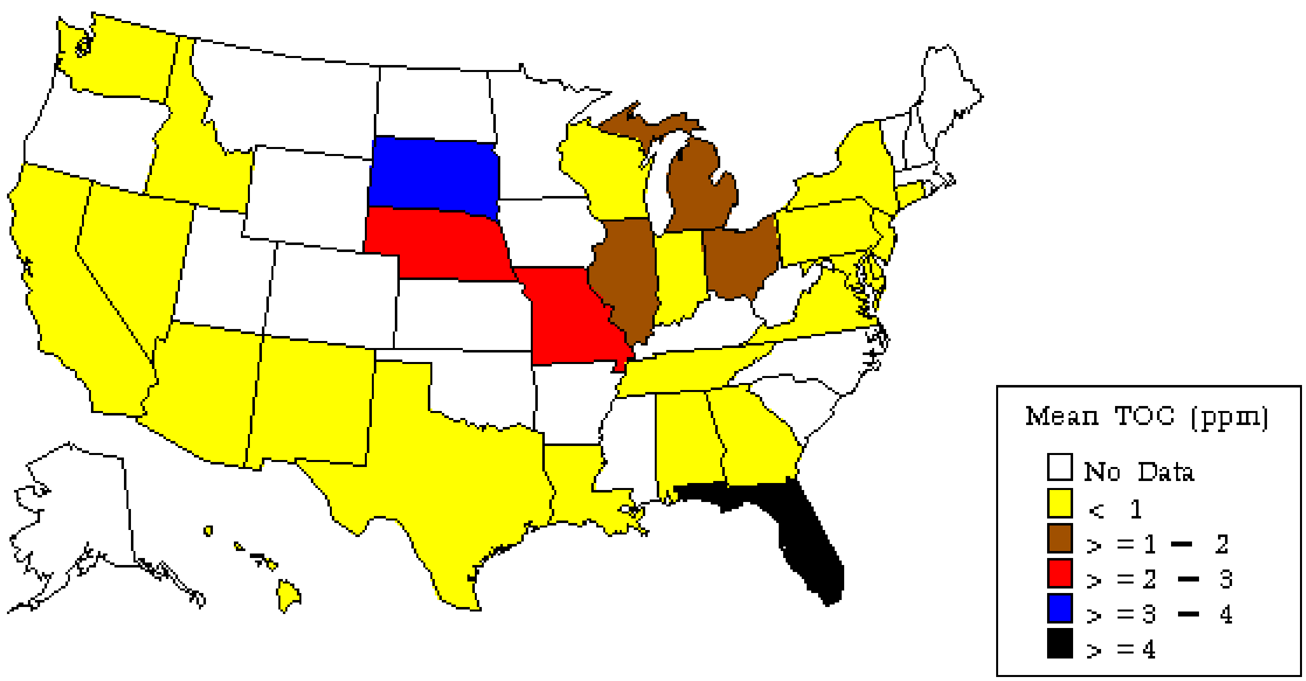
Groundwater TOC

III. Treatment Plant Influent D/DBP Questions

Question 1. What are the levels of DBP precursors?

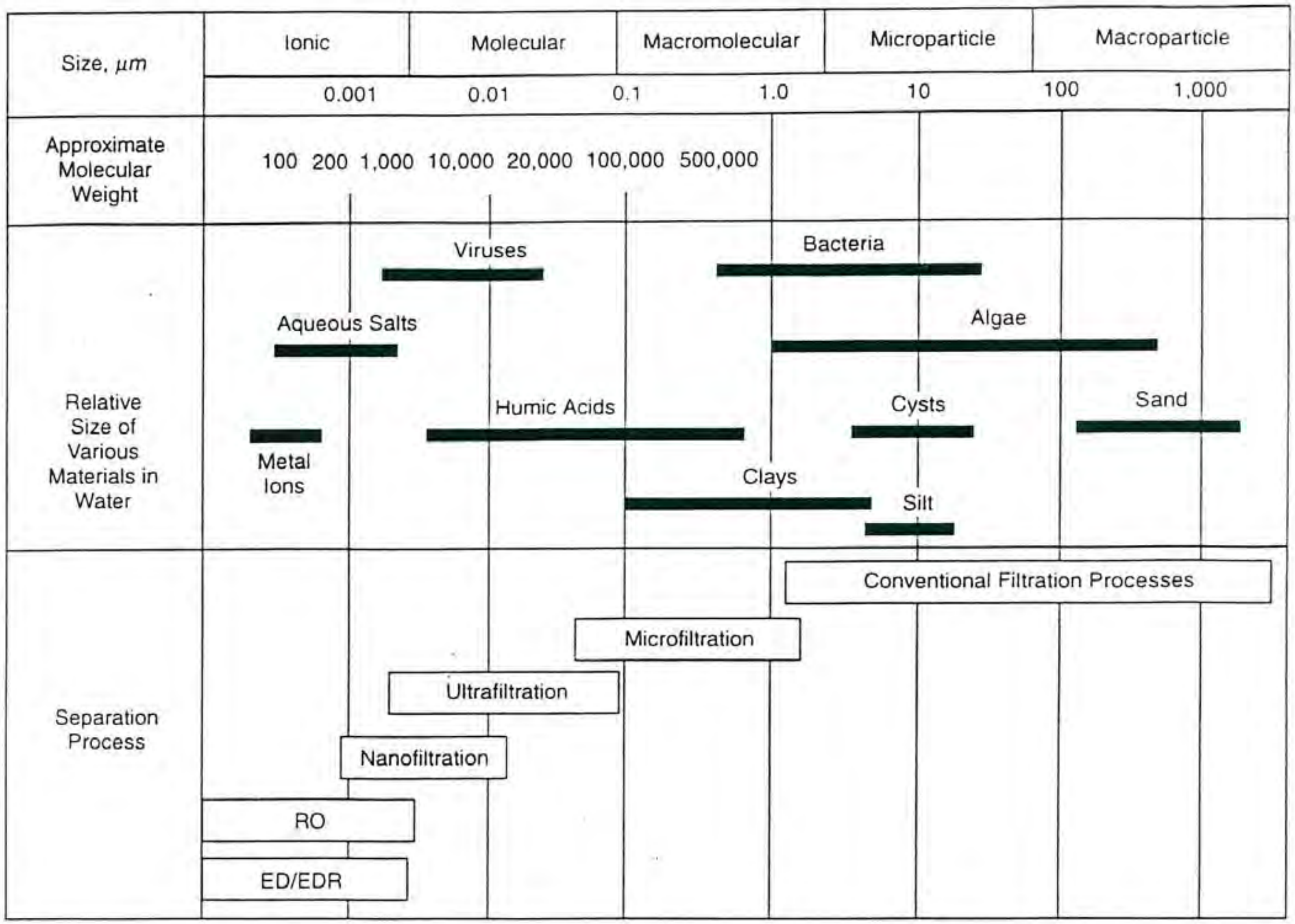
Mean Influent TOC by State — Plant Means of 12 Months

Ground Water Plants (n= 131)

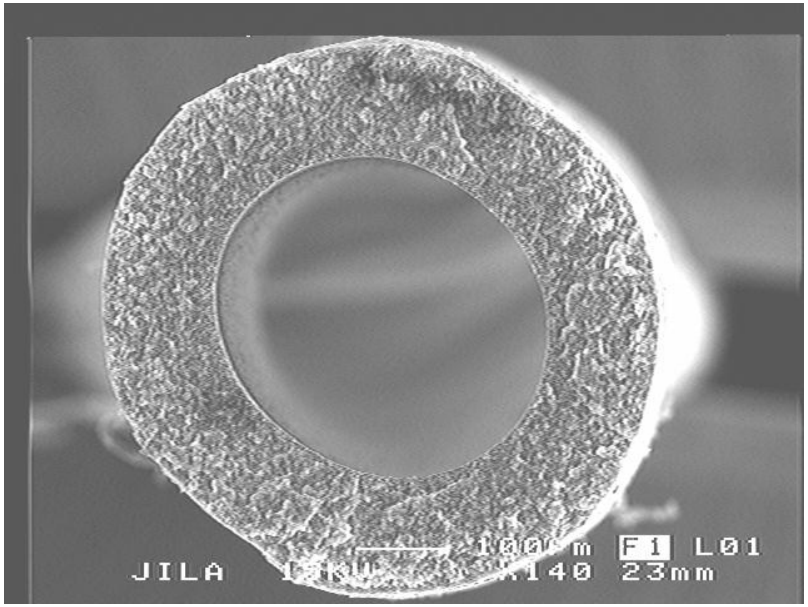


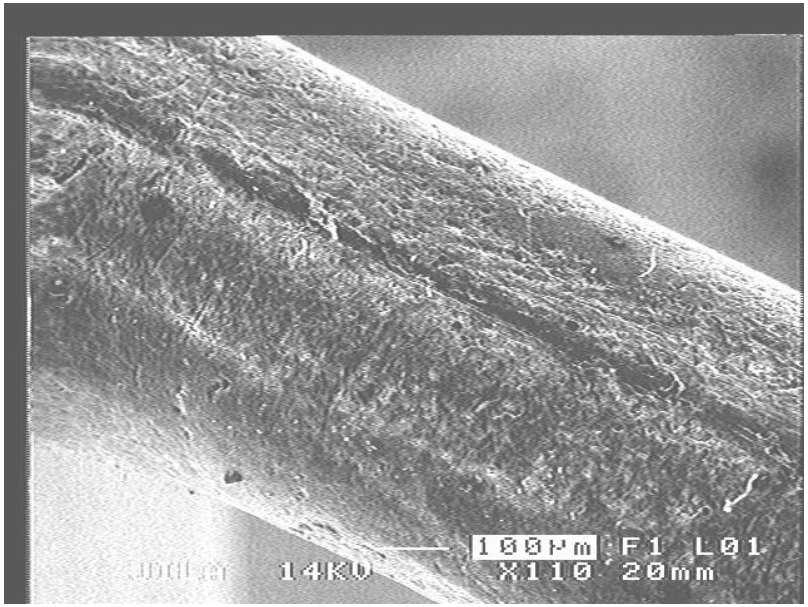
C1A3DMS.V3 —— G003JB3.SAS

Particle Removal Processes

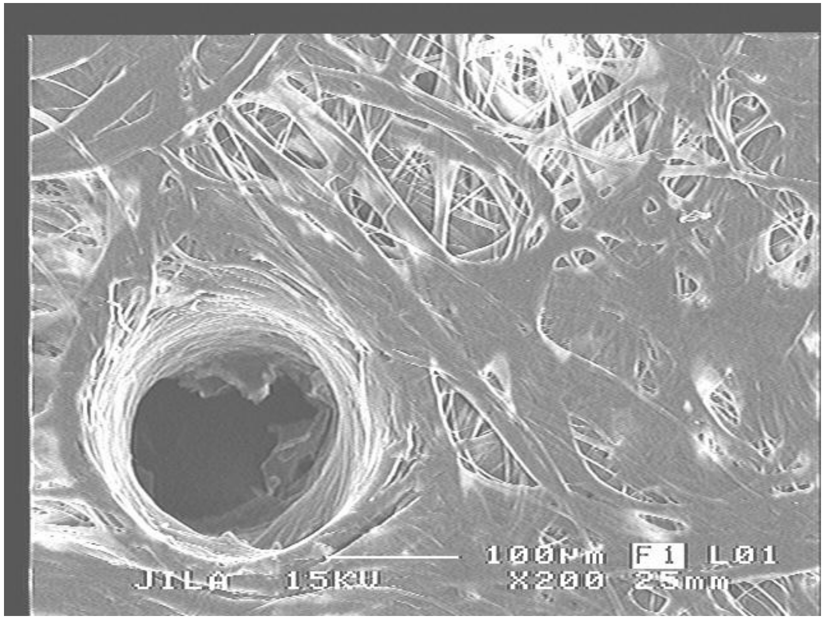


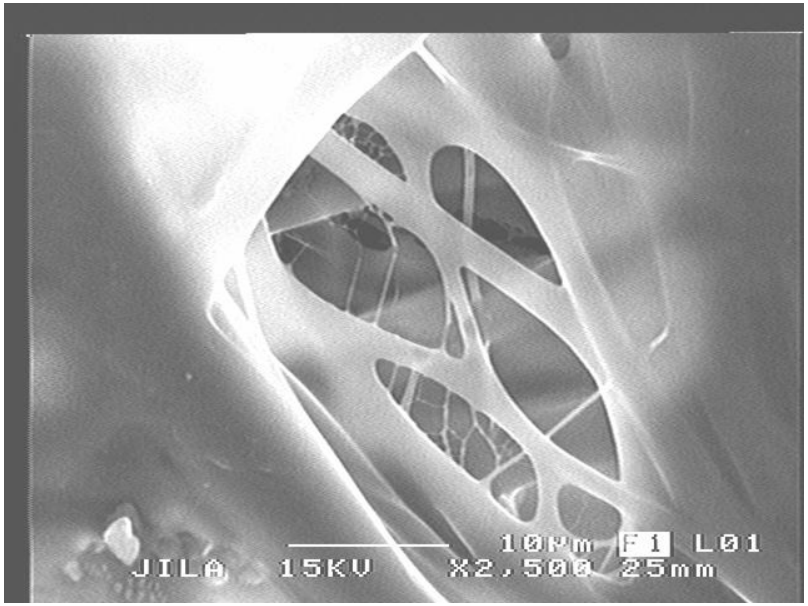
Microfiltration





Ultrafiltration





Toxic Tort Action

Lawsuit claims trihalomethanes were cause

154 women sue Chesapeake, Va. saying water caused miscarriages

CHESAPEAKE, Va. (AP)— An additional 129 women have filed lawsuits claiming the city's drinking water caused their miscarriages, bringing the total number of plaintiffs to 154. The women are seeking more than $1 billion.

The lawsuits allege that the city knowingly and/or negligently poisoned the women and their fetuses when the women drank, showered, bathed in, or used city water containing trihalomethanes, or THM.

more lawsuits are expected.

City officials have disputed the allegations and claimed that Chesapeake has stayed within the federal guidelines for safe drinking water.

The city also contends it cannot be sued because the lawsuits were filed after a two-year statute of limitations had expired, and that the city is protected by sovereign immunity. City officials have asked that any court proceedings be moved to another jurisdiction to ensure a fair trial by an

U.S. Water News, Sept. 2001

What Are the Critical Research Questions?

- Effect relative to other Effects
- Occurrence, Fate and Transport
- Treatment Effectiveness
- Suitable Operational Surrogates

Sorption & Transport of Hormonally Active Agents: Initial Laboratory Results

P. Suresh C. Rao & Linda S. Lee
Purdue University, West Lafayette, IN

Carl G. Enfield
NRMRL – USEPA, Cincinnati, OH

United States Environmental Protection Agency

Project Team

Purdue University: School of Civil Engineering

- Suresh Rao, Professor (Co-PI)
- Ajit Sarmah, Post-doc
- Nathan Haws, PhD student
- Ryan Hultgren, PhD student

Purdue University: Agronomy Department

- Linda Lee, Professor (Co-PI)
- Sylvie Brouder, Associate Professor
- Maurilio Oliveira, Post-doc
- Steve Sassman, Lab Manager
- Troy Strock, MS student

US EPA- NRMRL:

- Carl Enfield

US EPA- NRMRL:

- Andy Avel, Project Officer

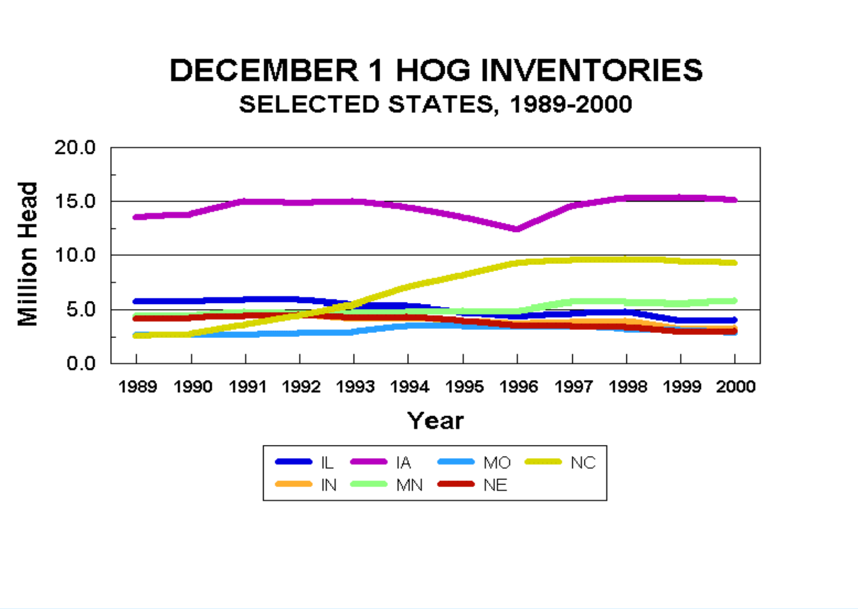
Project Funding Sources

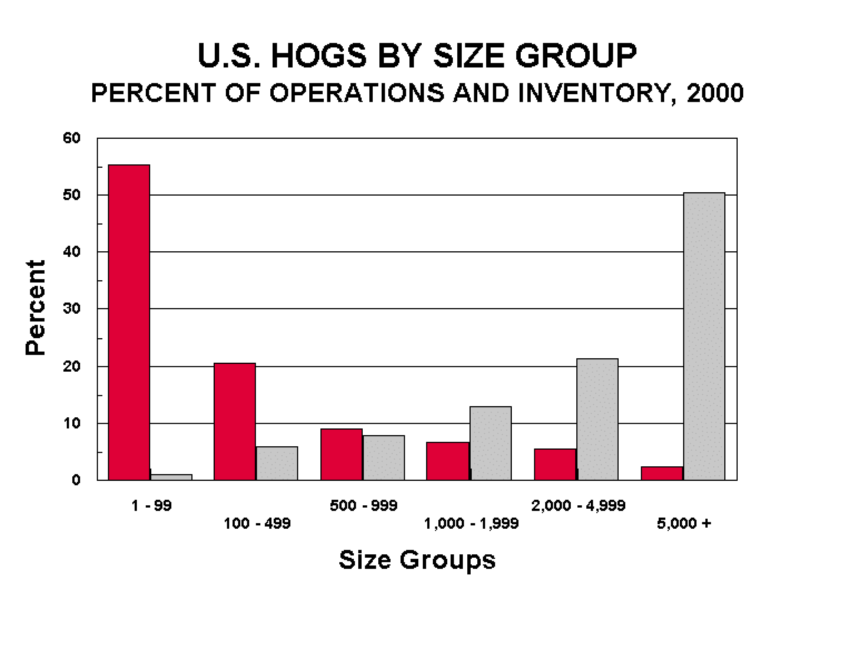
US EPA – NRMRL (2-yr project): (~ $100K for 2 yrs)

- Emphasis on EPA-designated Endocrine Disrupting Chemicals
- Primarily reproductive hormones
- National perspective

Purdue University (2-yr project): (~ $250K for 2 years)

- Emphasis on animal growth hormones
- Primarily confined animal feeding operations (swine)
- Indiana watersheds perspective





Significance of CAFOs in Indiana

- Number of hogs in IN constant since 1989 at ≈ 5 million head
- Small operations decreasing; large operations increasing.
- Steady decline in total number of hog operations since 1979 from 25,000 to 4,500.
- Over 60% have > 2000 hogs
- Now large amount of manure per area produced
- Large amount of growth hormones & antibiotics used

Initial Project Objectives & Tasks

- Identify major growth hormones used in swine CAFOs and reproductive hormones
- Conduct literature search for data on use, environmental fate processes, & analytical methods
- Conduct laboratory experiments to determine primary fate & transport parameters (K_{oc}, K_{ow}, solubility, $t_{½}$, etc.)
- Conduct column experiments to determine sorption, transformation and transport parameters under steady flow conditions in saturated media
- Monitor transport & fate of selected pharmaceuticals in tile-drained field plots receiving hog-manure application
- Modify existing models to evaluate field data

Hormonally Active Chemicals Selected

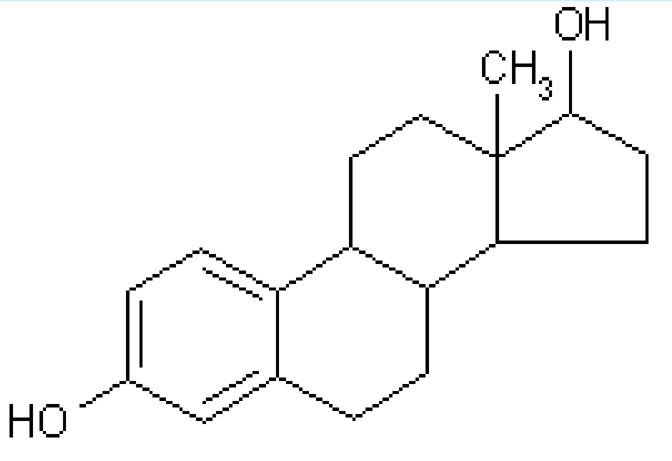
- Reproductive Hormones
  - 17β–estradiol (natural)
  - 17α–ethynyl estradiol (synthetic)
  - testosterone
- Animal Growth Hormones
  - carbadox
  - tylosin
  - tetracyclines (chlor- and oxy-)
  - bacitracin
  - bovine growth hormone (BGH)

Soils & Sediments Selected

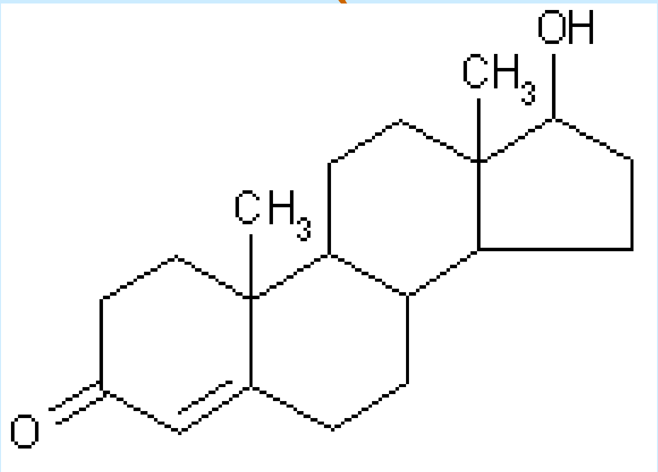
	%OC	%clay	pH
EPA Soils/Sediments			
EPA-1 (MN)	0.22	6	7.3
EPA-14 (WVA)	0.48	64	4.3
EPA-23 (IL)	2.38	69	7.1
Soils			
Bloomfield-3 (IN)	0.36	8	6.4
Drummer-1 (IN)	2.91	21	7.2
Toronto-4 (IN)	1.34	21	4.4
Chalmers-6 (IN)	1.20	16	6.5
Milford-21 (IN)	0.41	36	7.4
Eustis (FL)	0.40	2	5.5

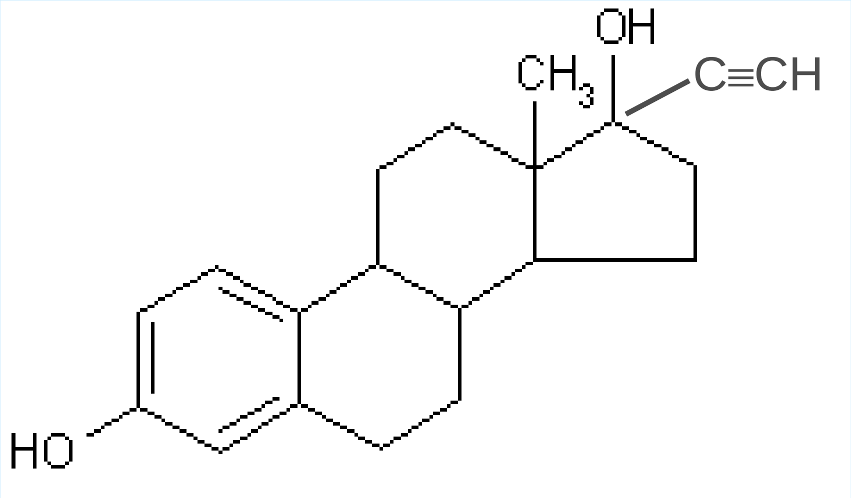
Estrogens & Androgens

17β-estradiol (272.4 g/mol)

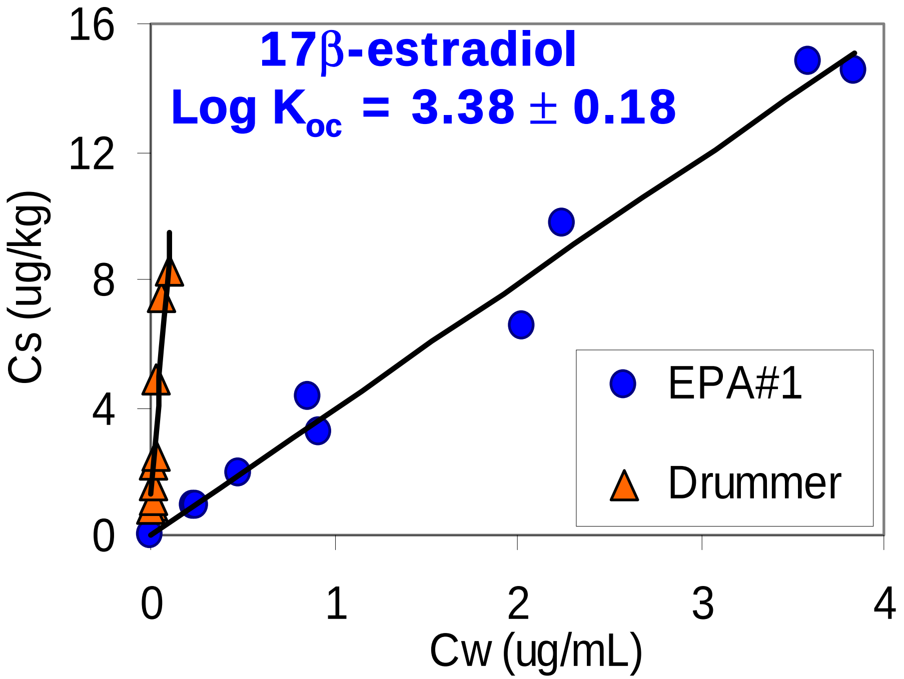


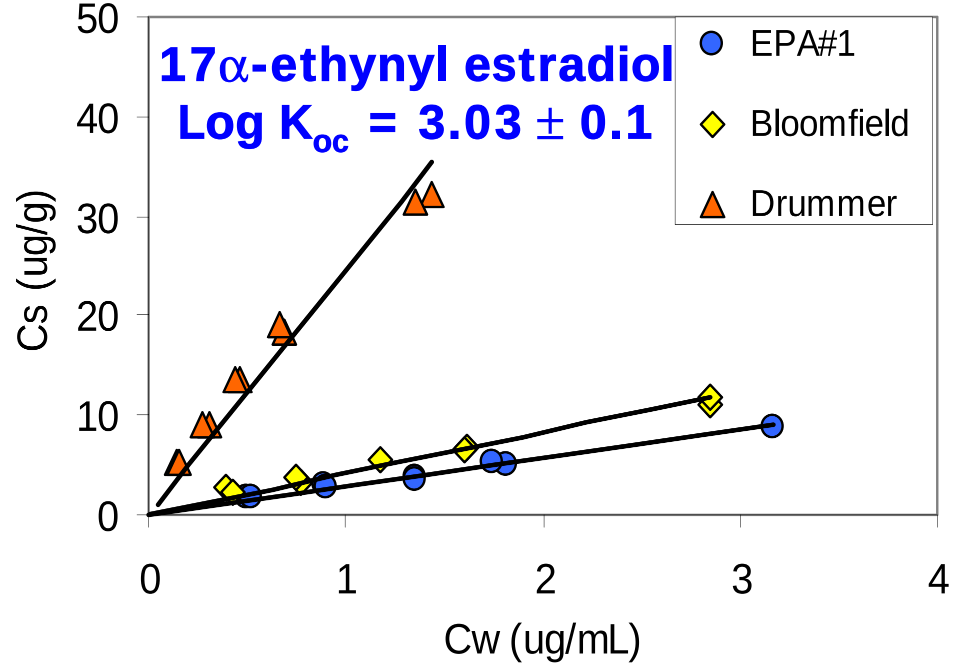
Testosterone (288.4

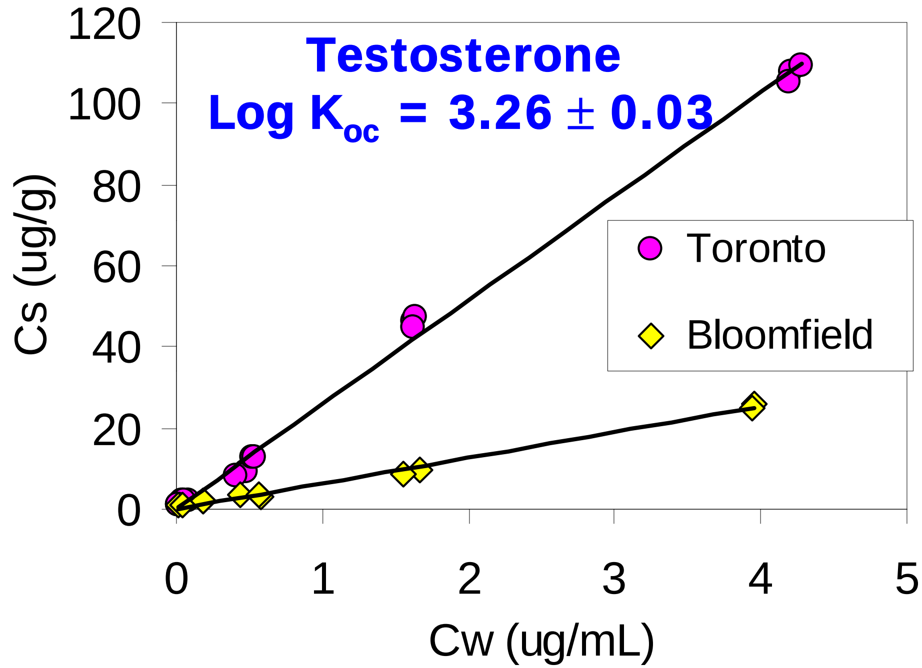




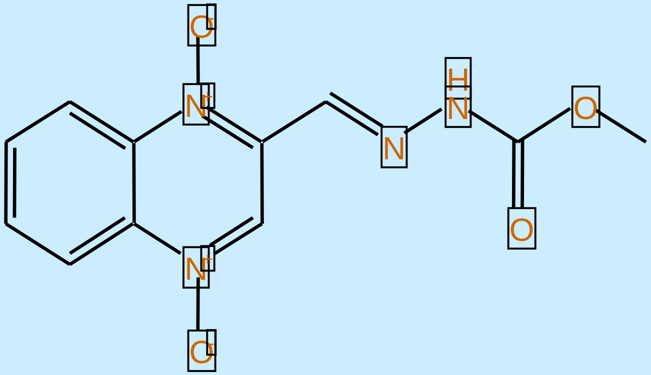
17α-ethynyl estradiol (296.4 g/mol)

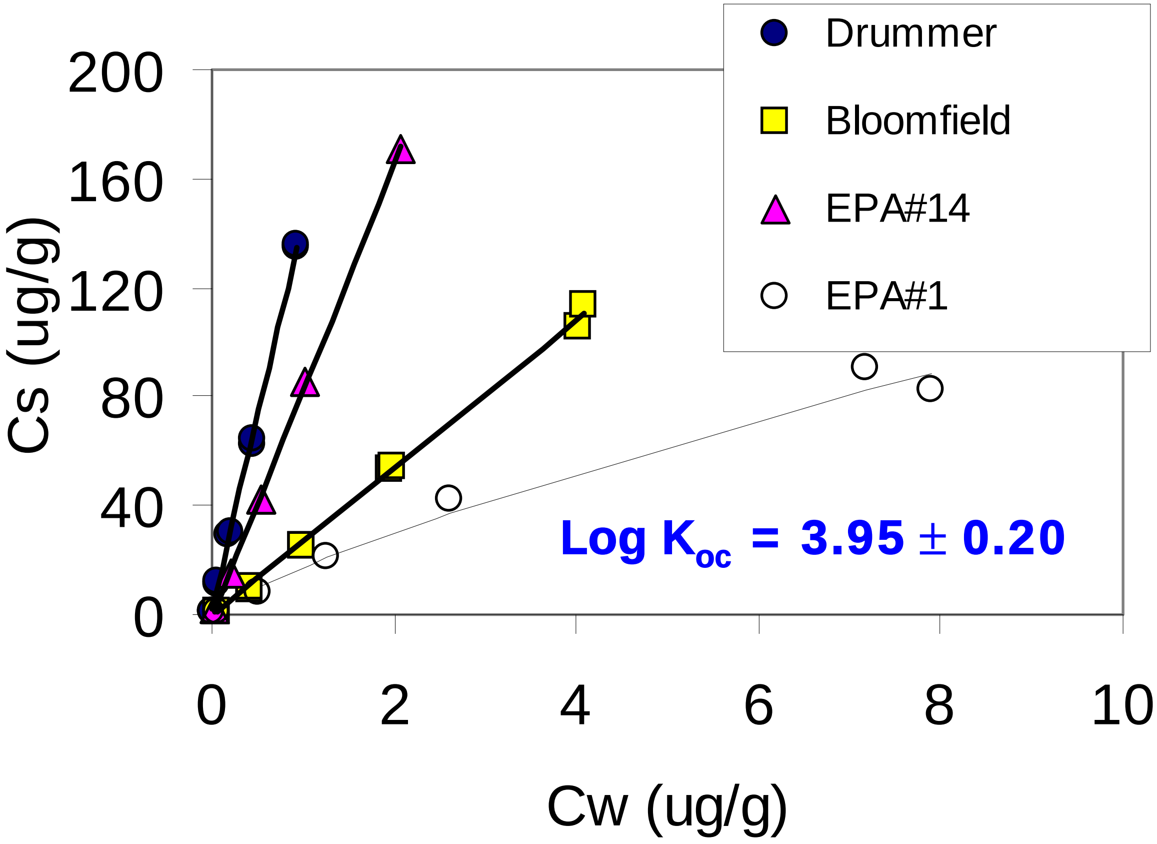

17β-estradiol
Log K$_{oc}$ = 3.38 ± 0.18
Cs (ug/kg)
Cw (ug/mL)
EPA#1
Drummer
16
12
8
4
0
0
1
2
3
4


17α-ethynyl estradiol
Log K$_{oc}$ = 3.03 ± 0.1
Cs (ug/g)
Cw (ug/mL)
EPA#1
Bloomfield
Drummer
50
40
30
20
10
0
0
1
2
3
4


Testosterone
Log K$_{oc}$ = 3.26 ± 0.03
Cs (ug/g)
Cw (ug/mL)
Toronto
Bloomfield
120
100
80
60
40
20
0
0
1
2
3
4
5

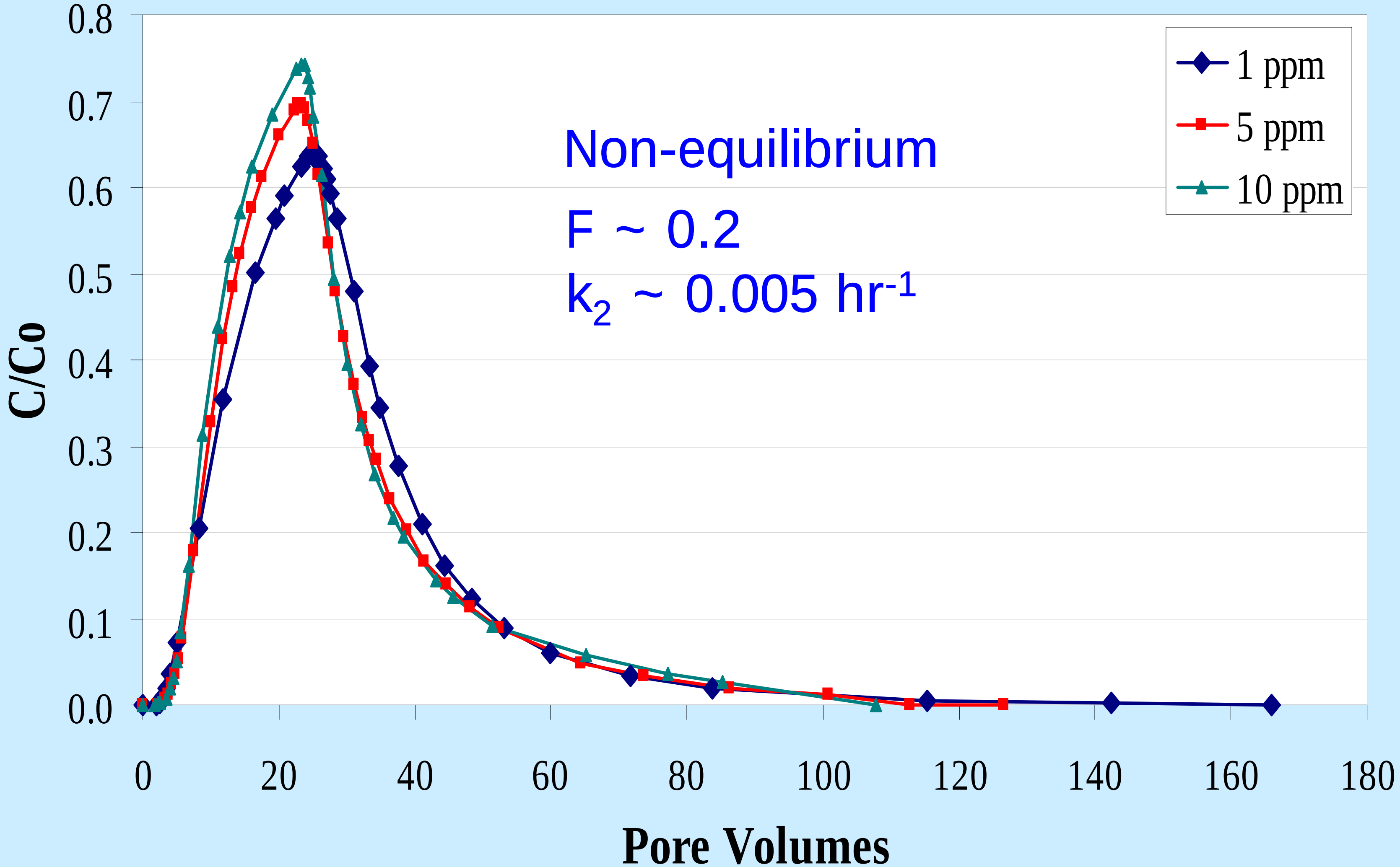
Carbadox



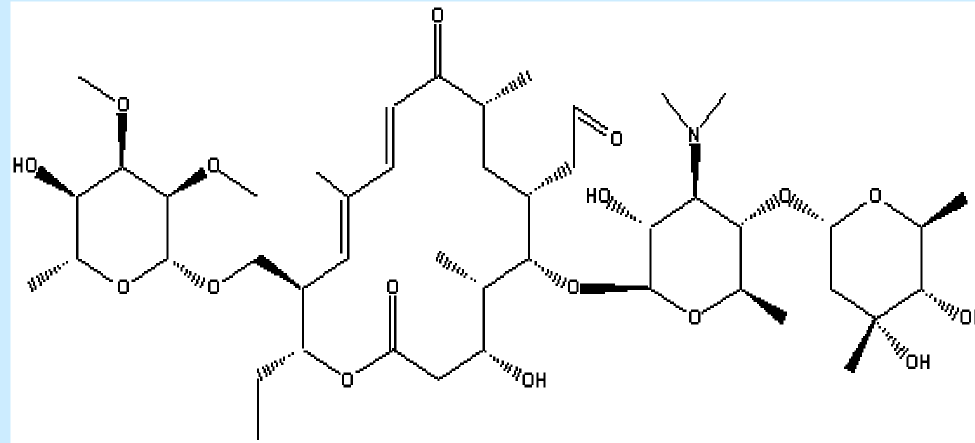


- Used in swine pre-starter feeds as growth promoter
- Aqueous solubility 51 mg/L
- Desoxy degradation product (in kidneys & liver) is carcinogenic, more polar & water soluble

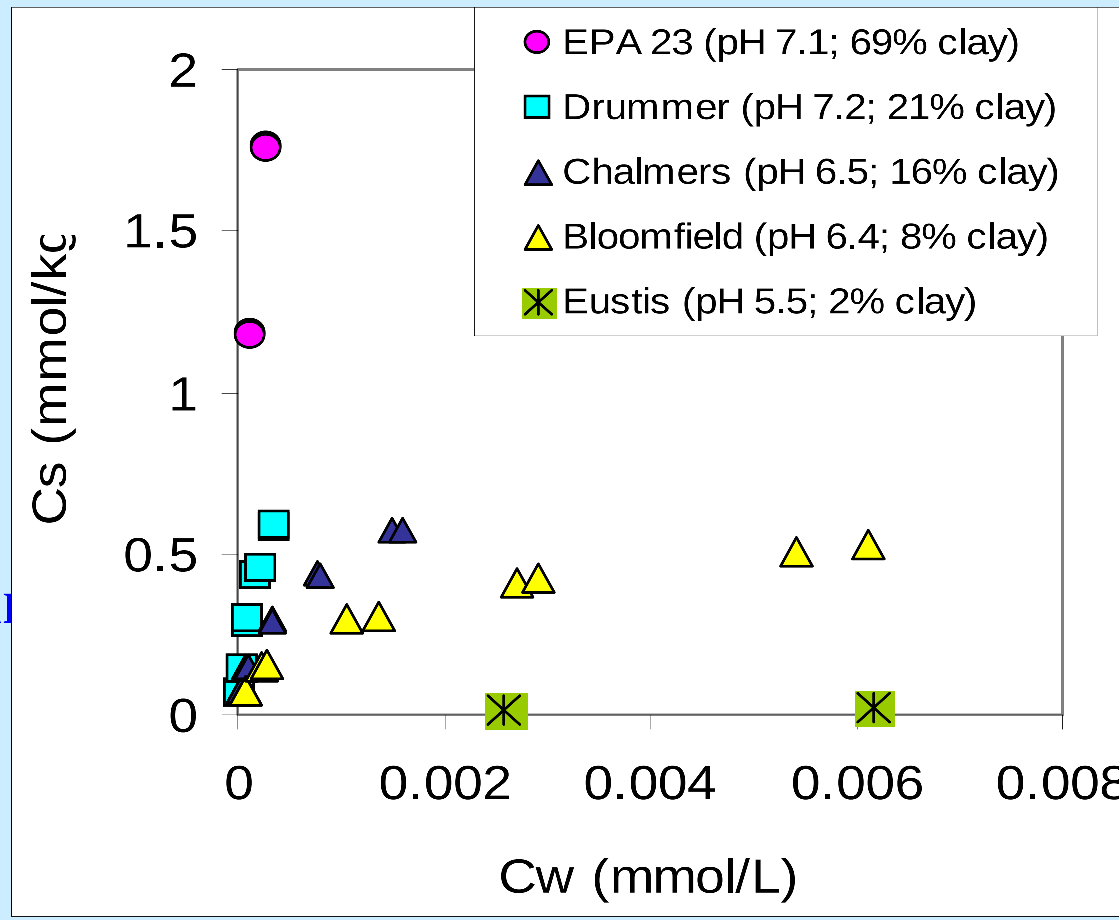
Carbadox on EPA-1 Soil Column: Concentration Effects



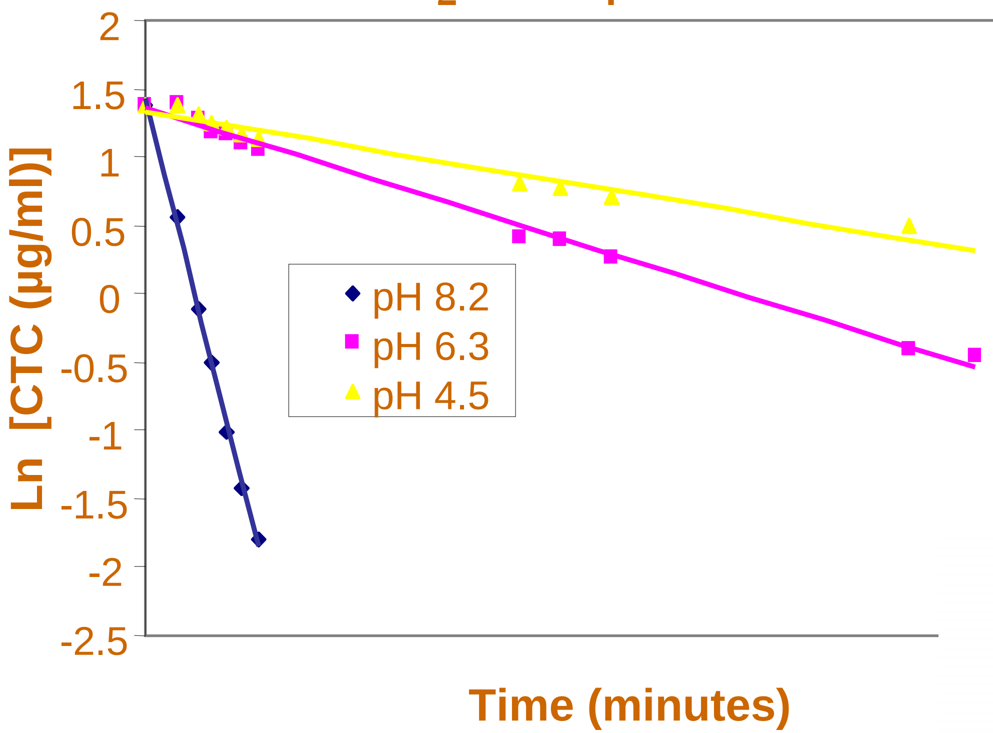
Tylosin (A)

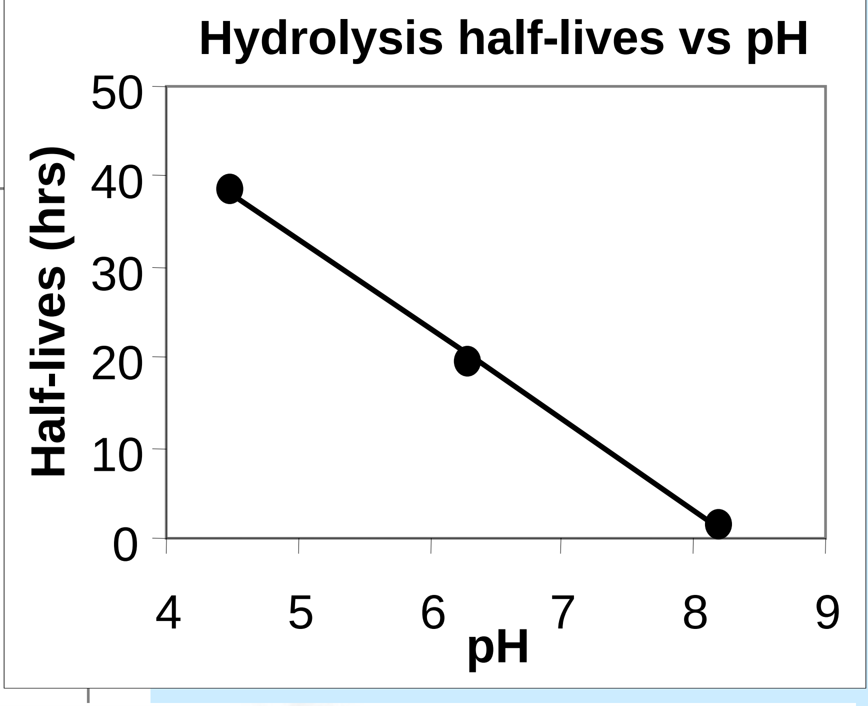


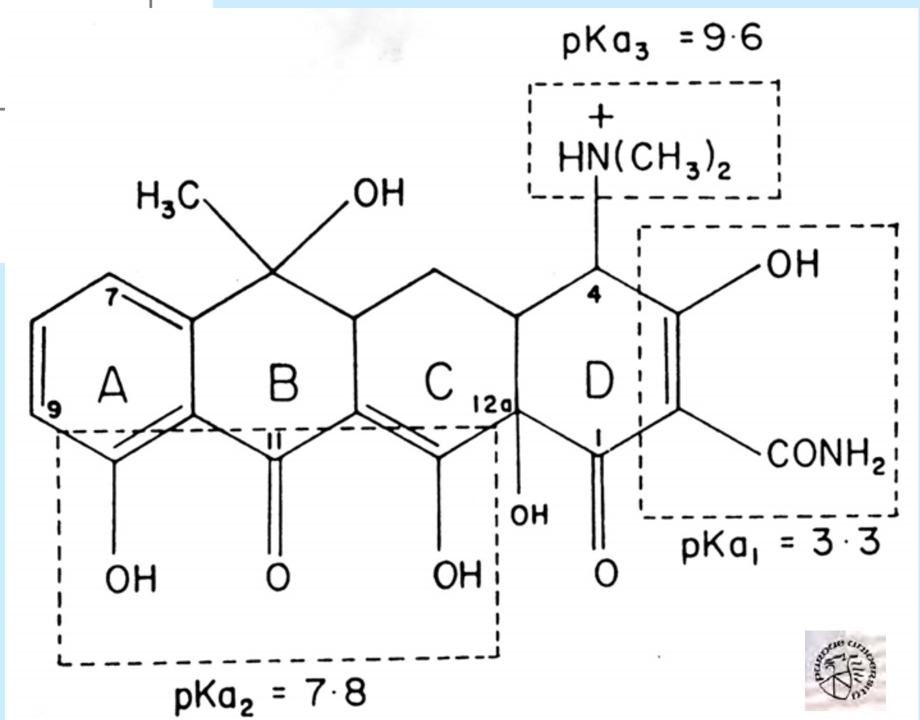
- $C_{46}H_{77}NO_{17}$ 916.1 g/mol
- Widely used in swine CAFOs
- Weak base - $pK_a = 7.1$
- $S_w \approx 5{,}000$ mg/L
- pH 4-9 relatively stable
  pH < 4, acid hydrolysis
  pH > 7 Aldol forms
- K_d: 2 to > 3000 L/kg
- At WQFS: up to 1 ppm i
  field-applied manure



Chlortetracycline Stability in Na_2HPO_4 buffer







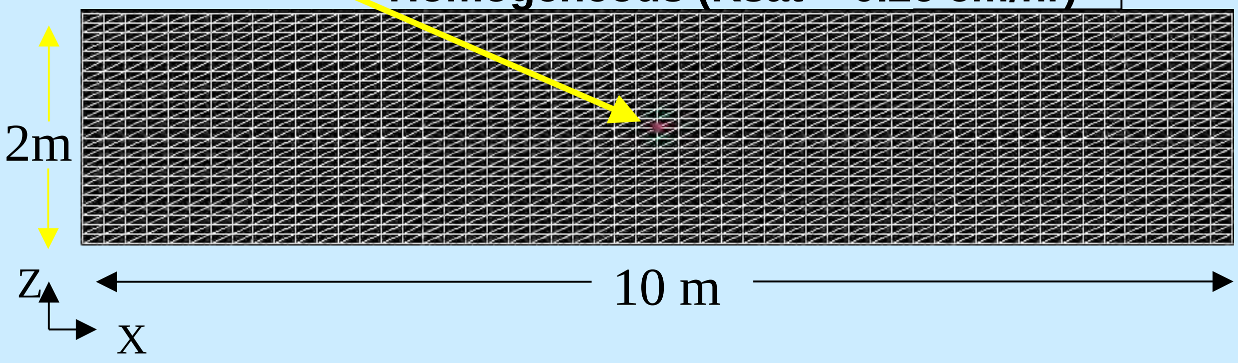
Sorption by Soils in 0.01 N $CaCl_2$:

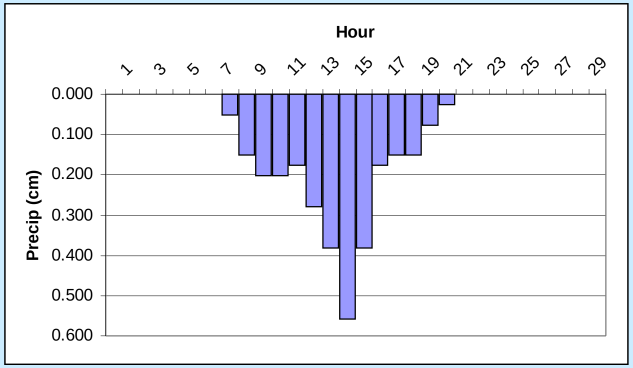
Single Point K_d values of 1000 to 1500 L/kg assuming sorption by difference estimated for 6 soils ranging in pH (2.5 – 6.4) and %OC (0.2 – 3%).

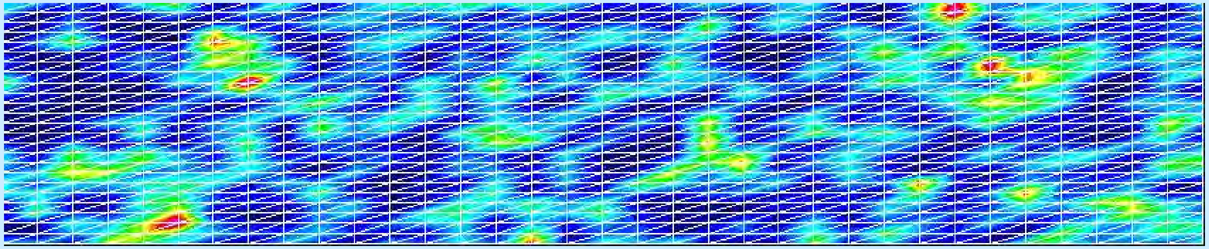
Preliminary Numerical Experiments Using HYDRUS-2D

Nodal Drain

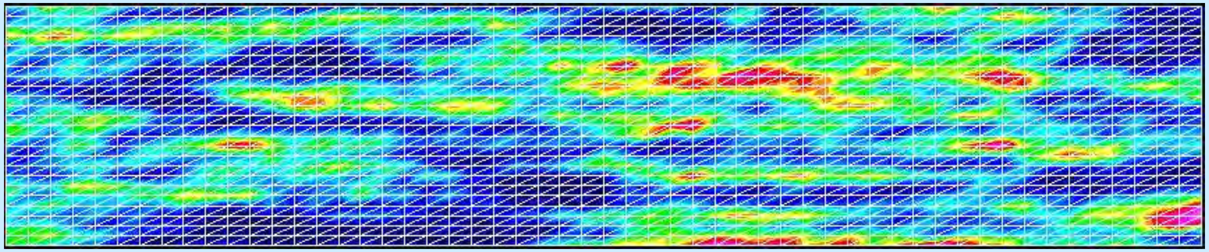
Homogeneous (Ksat = 0.26 cm/hr)



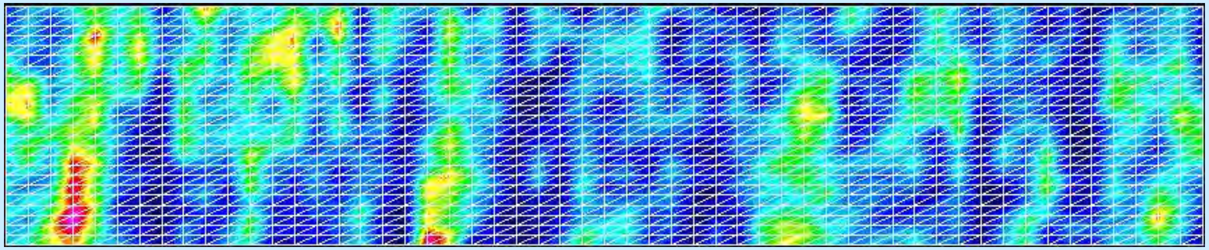




Isotropic

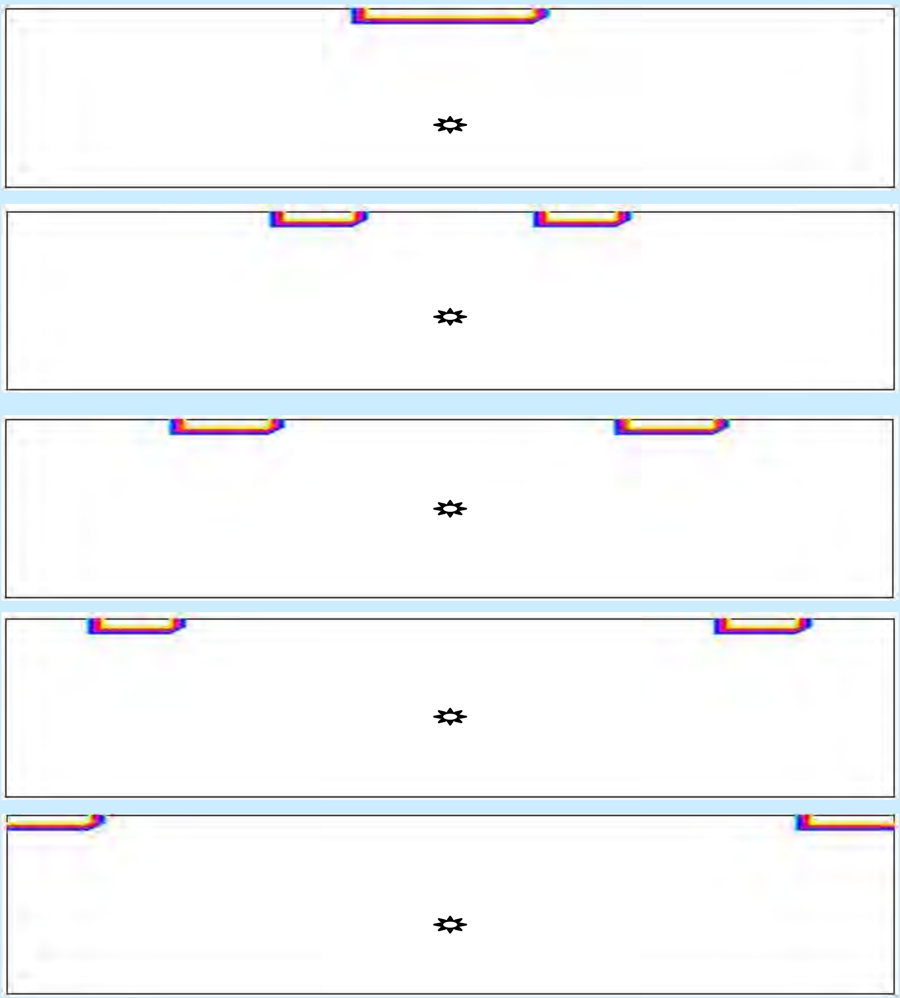


Horizontal Anisotropy

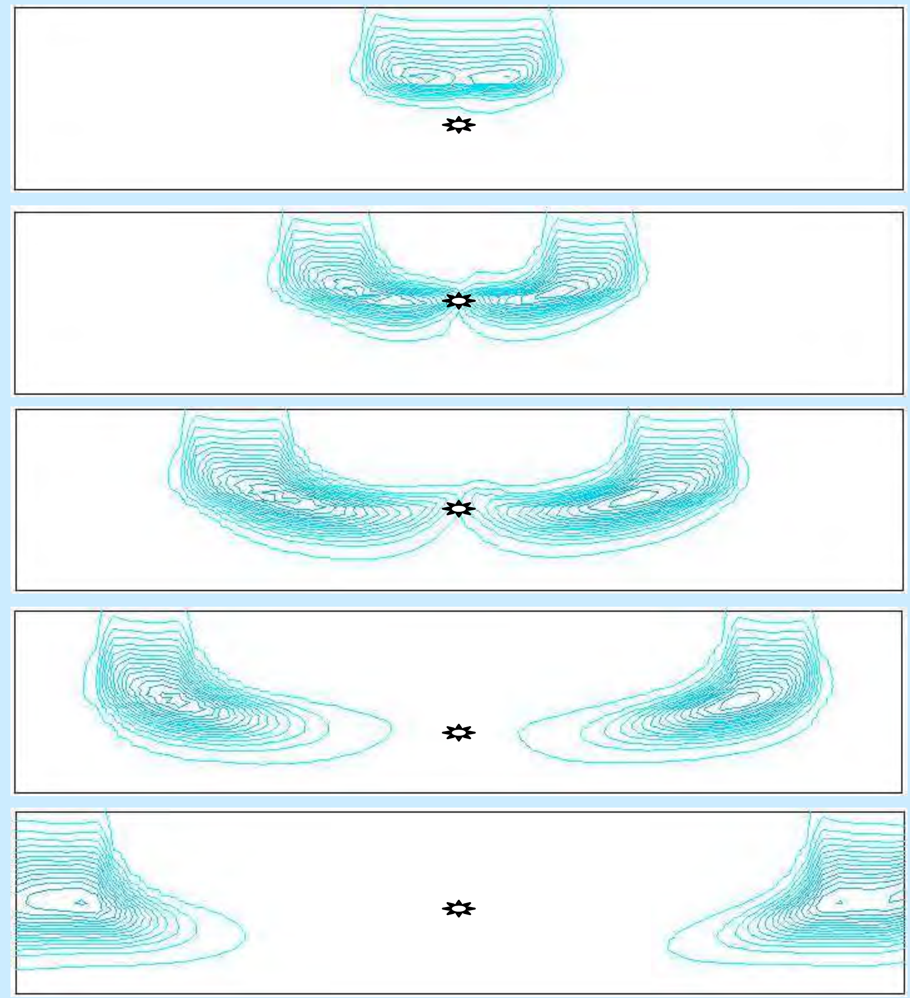


Vertical Anisotropy

HYDRUS-2D Simulation of Tracer Transport

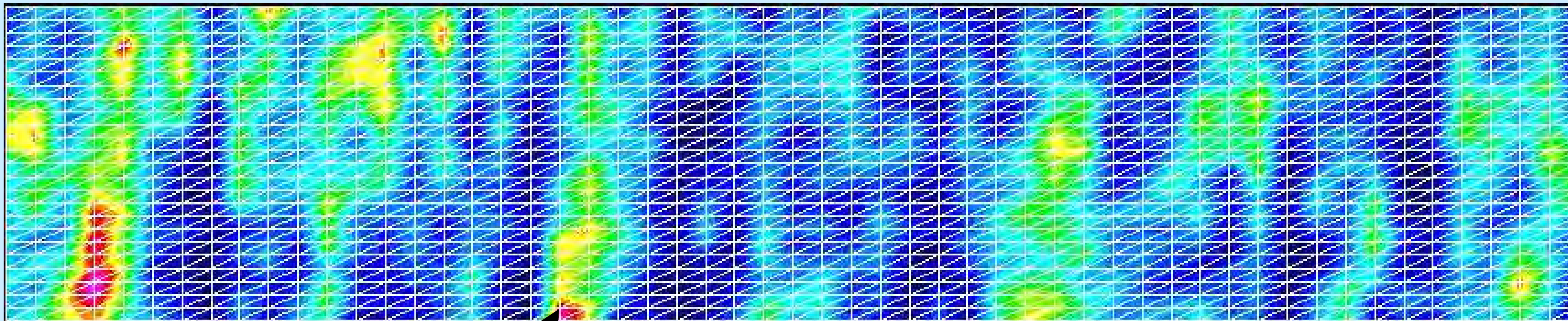


Initial distribution of five applied tracers



Distribution of five tracers at t= 8000 hrs

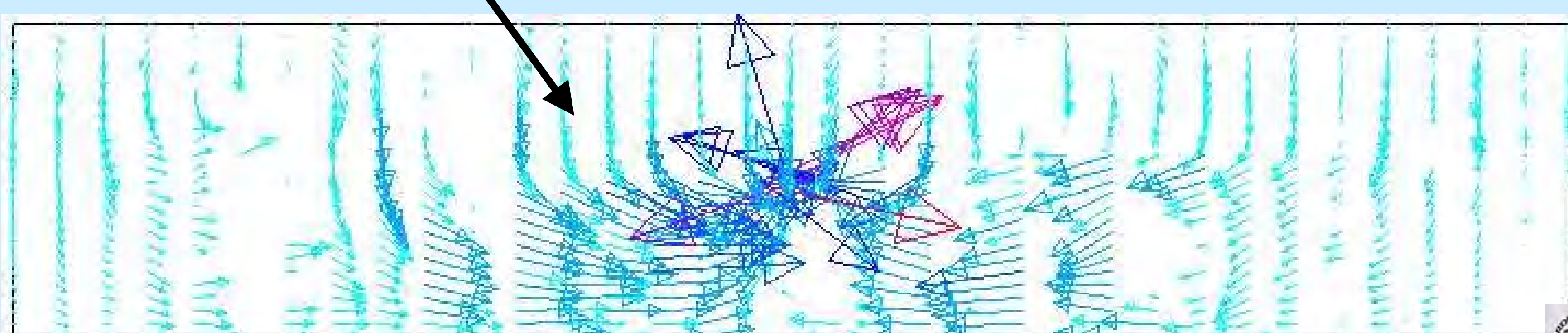
Conceptual Model: (Heterogeneous case)



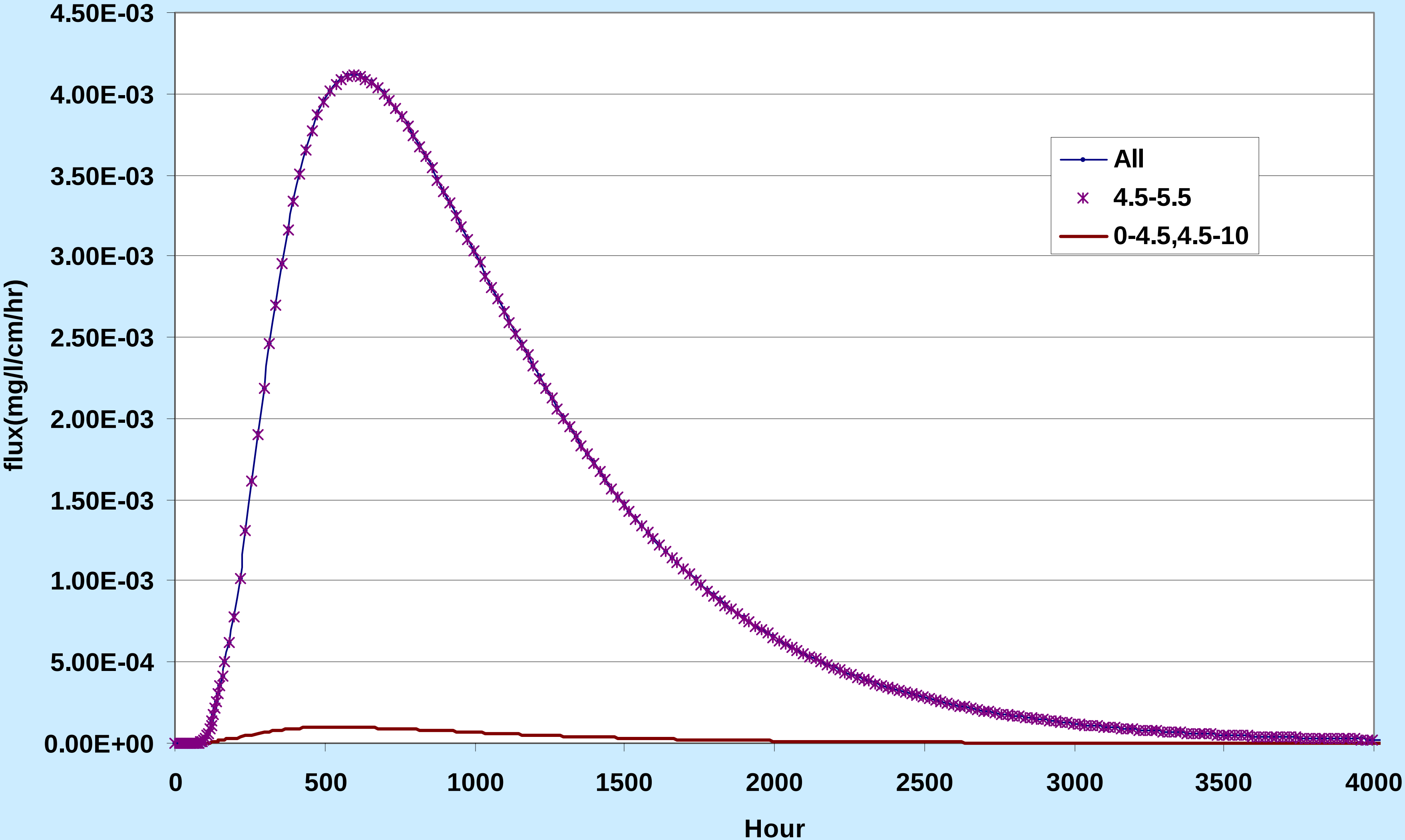
Vertical preferential pathways only effective in near-tile area

Ks Scaling Factors:

Std Dev.	1
x-correl.	10 cm
z-correl.	100 cm



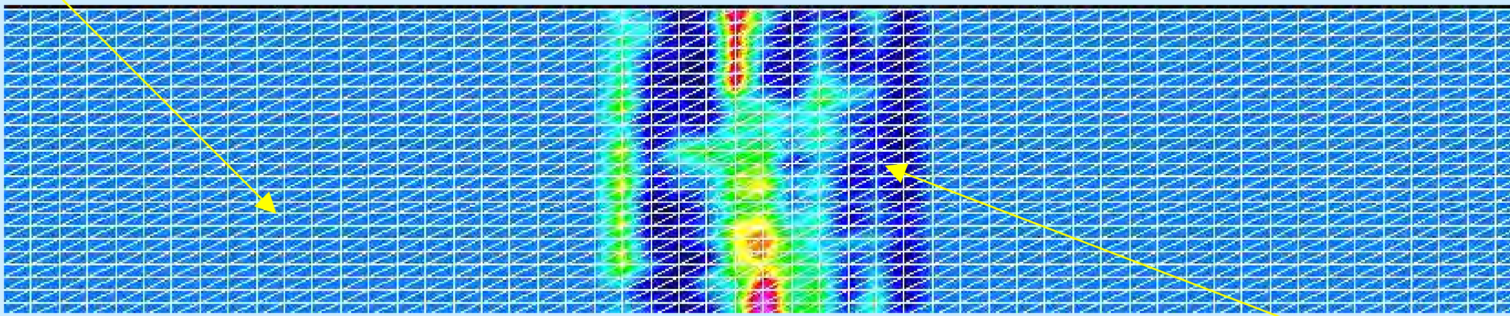
Recovery of Multiple Tracers



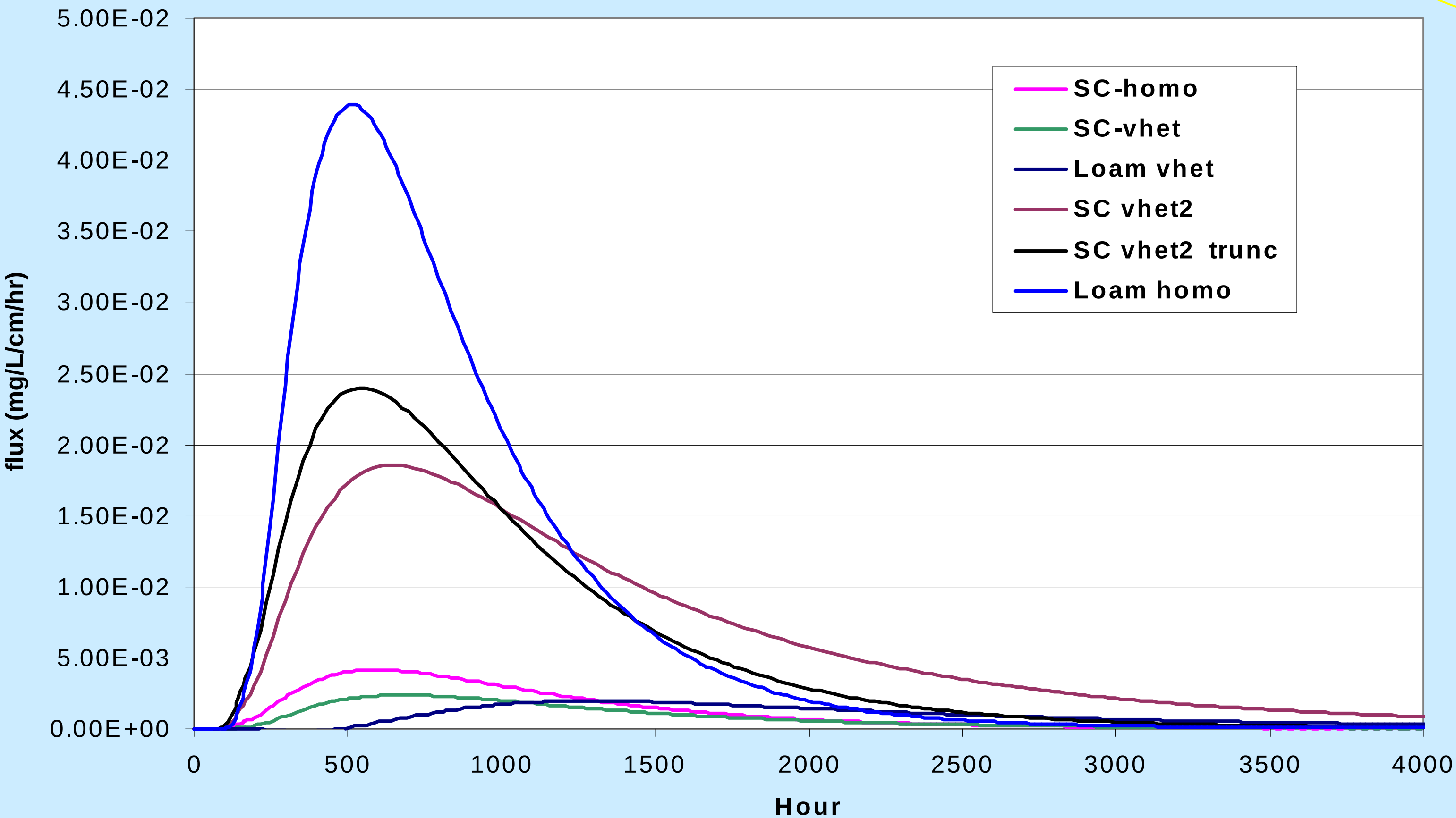
Note: Less than 0.1% of total applied tracers appear!

Single Event Transport Simulations (con't)

Homogeneous



Heterogeneous



Facilitated Transport of EDCs??

- Estrogens and testosterone have high sorption coefficients, suggesting limited leaching potential in soils.
- Facilitated transport of EDCs bound to mobile colloids (soil or manure) or complexed with DOC may be a major contributing factor to transport to surface & groundwater.
- Need to evaluate factors that contribute to preferential flow (e.g., macropores), and generation of DOC & mobile colloids.
- Desorption and bioavailability of bound EDCs??
- Field-applied manure at WQFS: 2% by weight of water-soluble organic material in a single batch equilibration (4g:20mL) in preliminary estimates.

Planned Activities

- Develop a comprehensive data set on environmental fate & transport parameters for the selected HAAs and selected soils/sediments (Purdue)

- Conduct lab column experiments (packed & undisturbed soil columns) to evaluate leaching potential and persistence under transient water flow conditions (Purdue)

- Conduct field experiments at the Purdue Water Quality Field Station (PWQFS) to evaluate tile flow water quality in plots receiving animal manure applications (Purdue & EPA)

Planned Activities (Continued)

- Develop a transport model to describe HAA transport data from soil column studies (Purdue & EPA)
- Develop a Lagrangian numerical simulator for describing field data from the tile-drained plots at the Purdue WQFS (Purdue & EPA)
- Identify Indiana field sites at which surface water quality can be monitored for HAAs (Purdue)
- Collect water samples near CAFOs and from impacted streams for HAA analyses (Purdue) and bioassays (EPA)

Residential Indoor Air and Dust Measurements of Phthalates and Other EDCs

Ruthann Rudel, Silent Spring Institute

US EPA Effective Risk Management of Endocrine Disrupting Chemicals

Cincinnati, Ohio -- January 29, 2002

Searching for Preventable Causes of Women's Diseases

Collaborators and Funders

- Silent Spring Institute
  - Ruthann Rudel and Julia G. Brody
- Harvard School of Public Health
  - Jack Spengler and Jose Vallarino
- Southwest Research Institute
  - Dave Camann, Paul Geno, Alice Yau, Michelle Ortiz

Funding from Massachusetts Department of Public Health, Boston Affiliate of the Susan G. Komen Foundation

Why an exposure study?

Exposure assessment is weak link in:

- Environmental epidemiology
- Risk assessment, esp. mixtures, indoor air
- Endocrine disruptors research

See *EHP* Dec. 2000, GAO reports, EPA EDSTAC, NRC Report on Hormonally Active Agents, NTP-CERHR Phthalate reports . . .

Why an exposure study?

- Widespread concern about environmental pollutants and breast cancer
- EDCs and animal mammary carcinogens are promising directions for study
- Exposure data can help to
  - ID chemicals and mixtures with common or high exposures as priorities for research and regulatory policy
  - ID highly exposed populations
  - ID major sources of exposure

To address these needs . . .

we developed and applied new methods to detect a broad range of compounds identified as hormonally active agents or animal mammary carcinogens and applied them to indoor air and dust samples

Study Design (*n=120 homes*)

	Number of Target Compounds				
Chemical Group	**Dust**	**Air**	**Urine**	**Interview**	**GIS**
Pesticides	~40	~40	~15	+	+
Alkylphenols	~7	~7	—	~	—
Phthalates	~10	~10	~5	~	—
Other estrogenic phenols	~10	~10	—	—	—
PCBs, PAHs	~6	~6	—	~	—
Parabens	~3	~3	—	—	—
Misc.	~10	~10	—	—	—
Estrogenic activity (E-SCREEN bioassay)	—	+	—	—	—

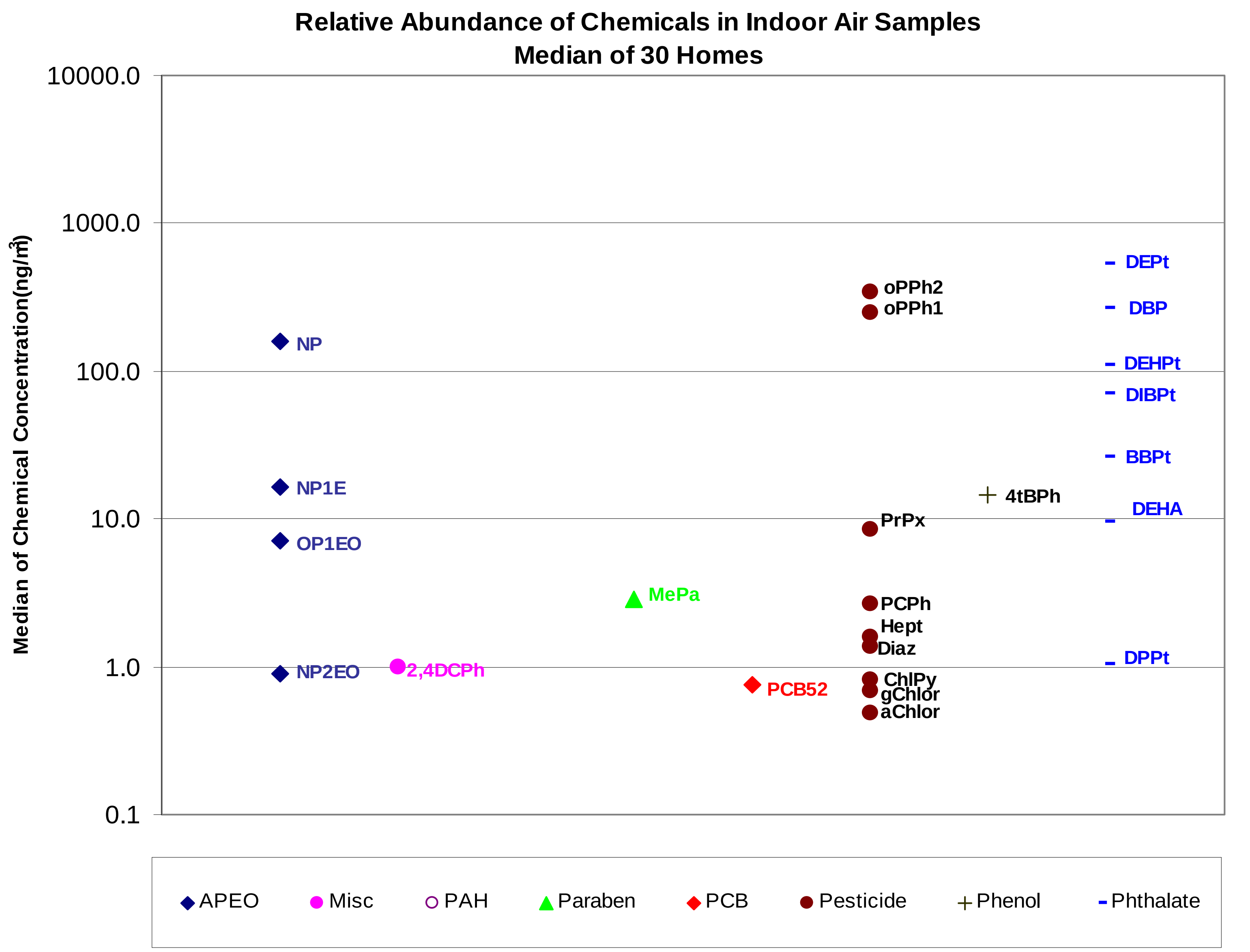
Methods

Sample collection and analysis

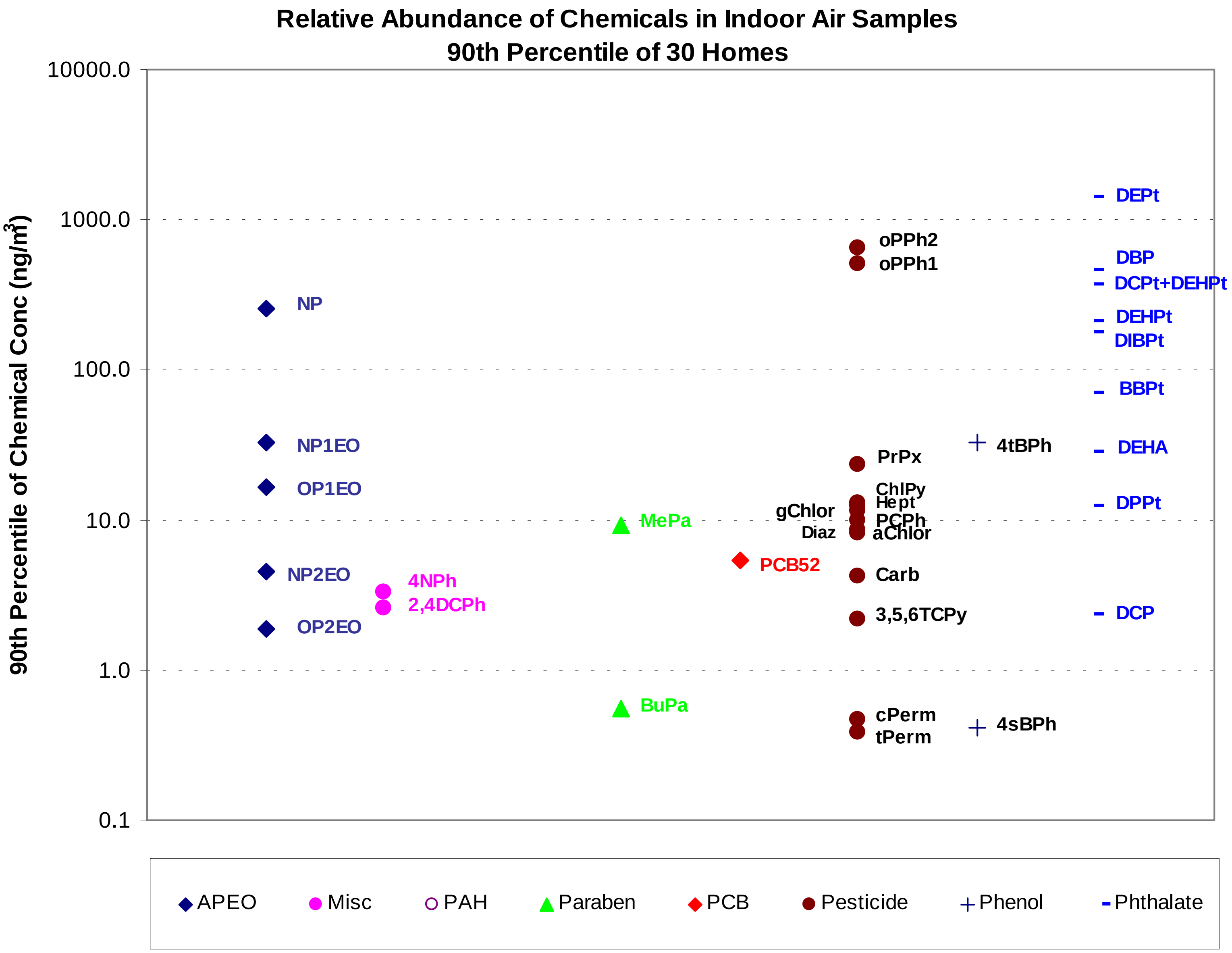
- Dust from living area using teflon crevice tool, collected into cellulose thimble, sieved to < 150 micron
- 24-hour air sample using high-vol pump, URG cartridge with quartz filter, PUF, XAD2, particles < 5-10 micron, ~ 13 m^3
- Two extraction/analytical methods (Rudel et al. 2001, *JAWMA* 51:499-513); phenols extracted with DCM, derivatized, GC/MS-SIM; neutrals Soxhlet extracted in ether/hexane, GC/MS-SIM.

Subject selection

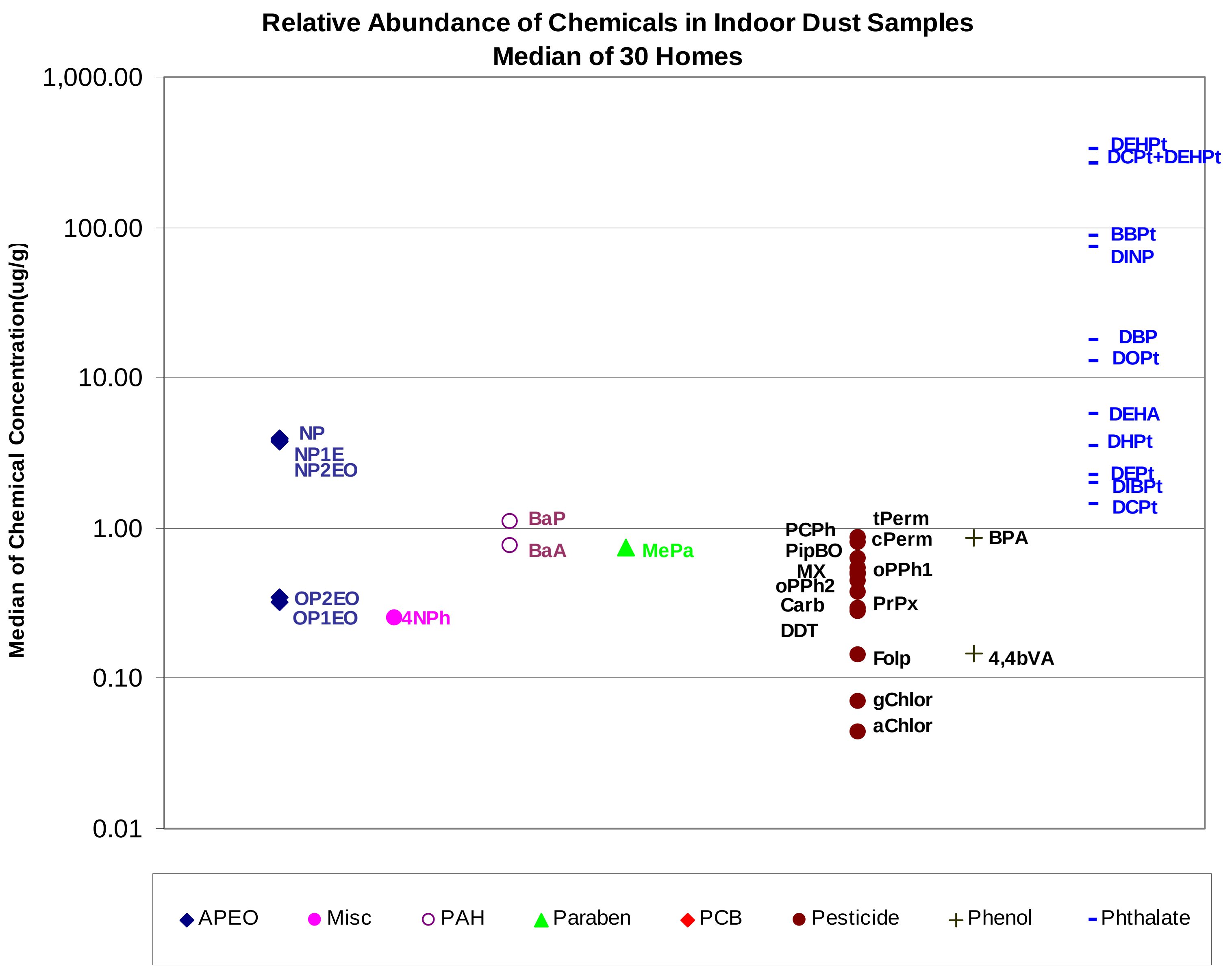
- Subset of women in case-control breast cancer study, over 65 years old, in current home > 10 years, oversampled high and low self-reported pesticide users.



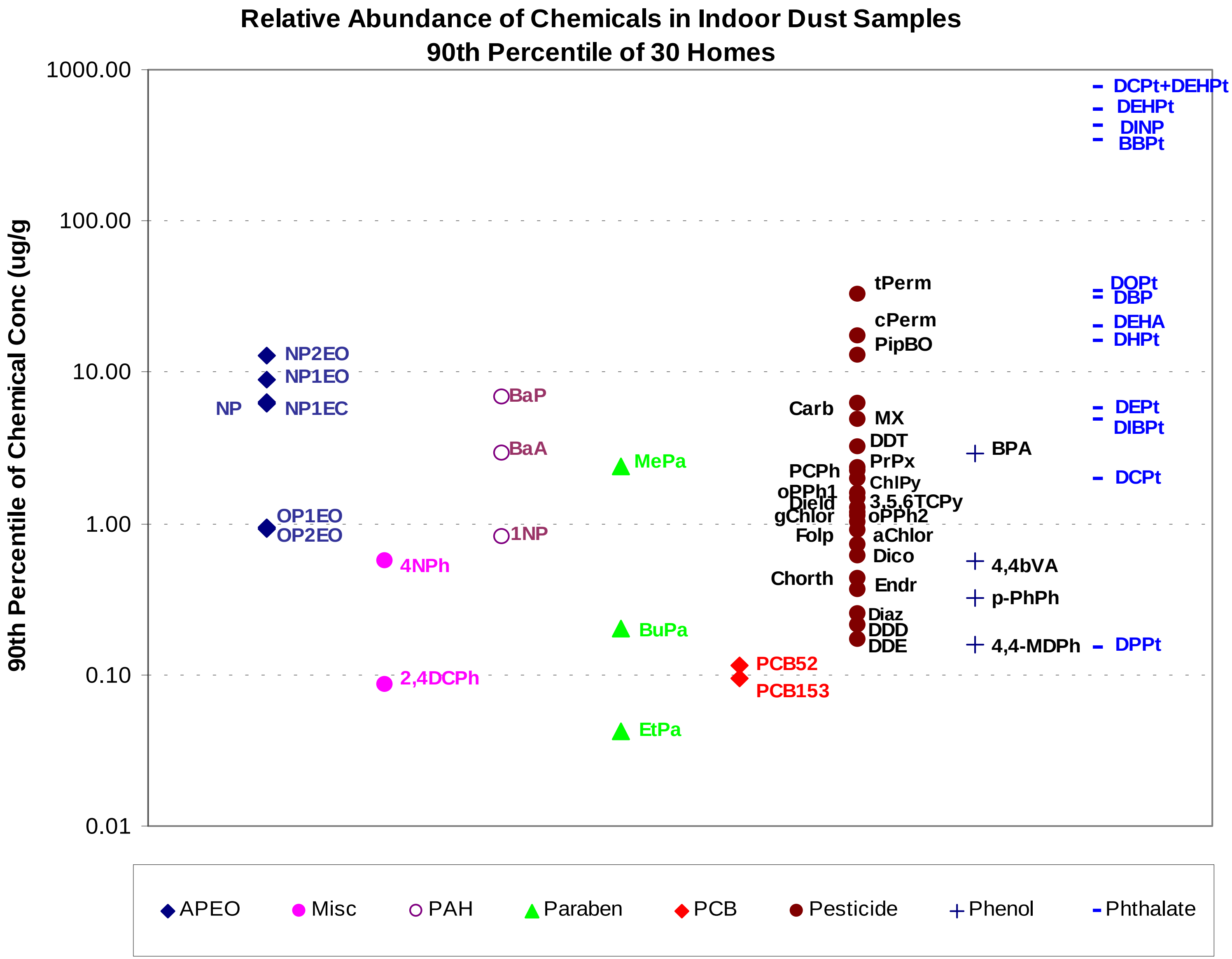
Silent Spring Institute



Silent Spring Institute



Silent Spring Institute



Silent Spring Institute

Pesticides detected in at least . . .

50% of homes:

o-phenylphenol
permethrin
piperonyl butoxide
carbaryl
methoxychlor
heptachlor
4,4'-DDT
propoxur
pentachlorophenol
chlorpyrifos
chlordane
folpet
diazinon

10% of homes:

All those plus

bendiocarb
3,5,6-trichloro-2-pyridinol
dieldrin
dicofol
chlorothalonil
endrin
4,4'-DDD
4,4'-DDE

Classes of chemicals detected, in order of relative abundance

Air

- phthalates (esp. DEP, DBP)
- *o*-phenyl phenol
- nonylphenol, APEOs
- pesticides
- 4-t-butyl phenol
- methyl paraben
- PCB 52

Dust

- phthalates (esp. DEHP)
- nonylphenol, APEOs
- pesticides
- PAHs
- bisphenol A
- parabens
- PCBs

Comparison with EPA Region 9 PRGs

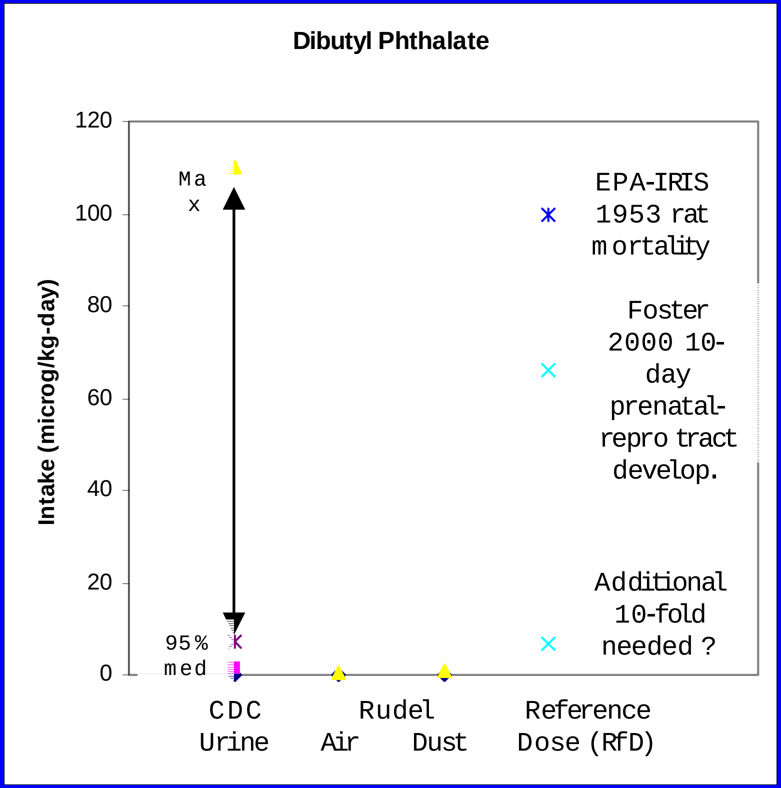
Compounds exceeding residential soil PRGs

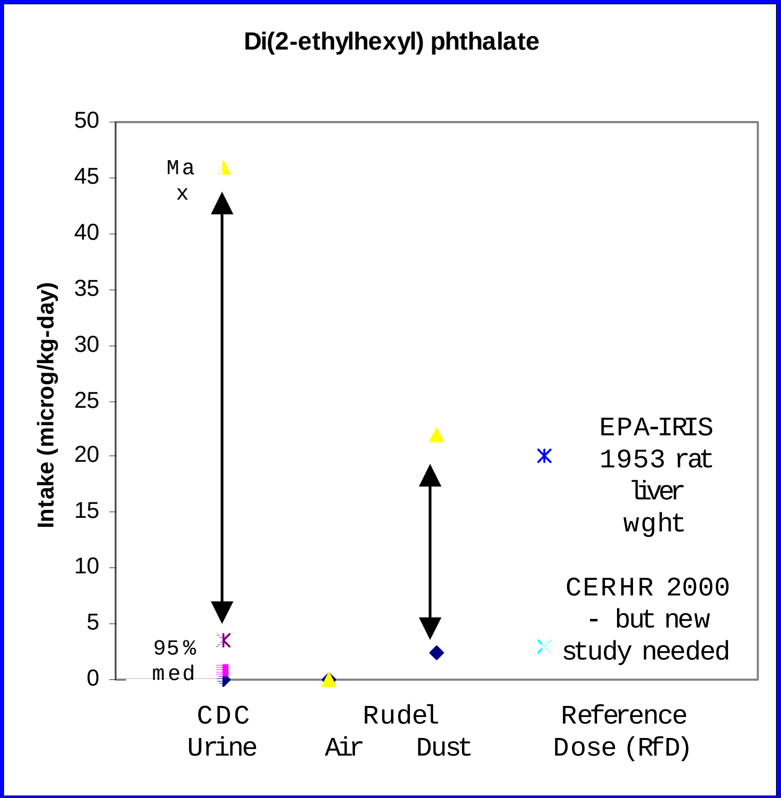
- Benz(a)pyrene
- Benz(a)anthracene
- PCB 52, 105, 153
- Heptachlor
- Chlordane
- Pentachlorophenol
- Dicofol
- Dieldrin
- DDT
- Bis(2-ethylhexyl)phthalate

Compounds exceeding air PRGs

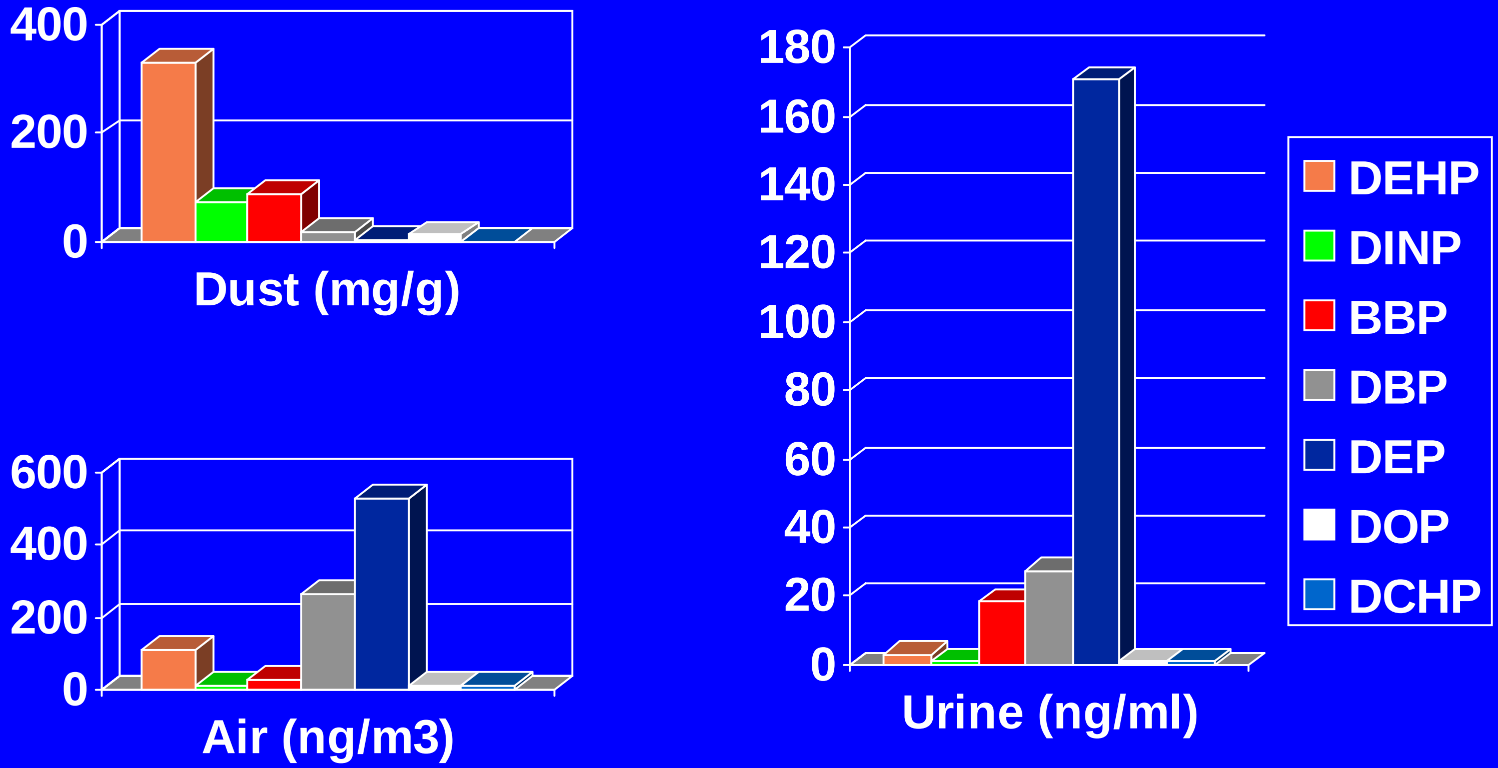
- Chlordane
- Heptachlor
- PCB 52

Exposure Levels-DBP, DEHP





Relative Abundance-Phthalates



From CDC National Report Card, 2001

Implications

A large number of toxicologically important chemicals (EDCs/MCs) are widespread in indoor environments

- Exposure data helps prioritize chemicals and mixtures for testing; identify major sources of exposure
- Need to understand health effects in order to limit exposure to the most important agents

www.SilentSpring.org

Welcome
Gregory Sayles, NRMRL
EDC Workshop January 29, 2002

Welcome to the CD-ROM version of the workshop, Effective Risk Management of Endocrine Disrupting Chemicals. This workshop was held in January 2002 in Cincinnati and sponsored by EPA's Office of Research and Development and EPA's National Risk Management Research Laboratory.

Hello, my name is Greg Sayles. I lead the risk management portion of EPA's endocrine disrupting chemicals research program.

To date, most research on endocrine disrupting chemicals (EDCs for short) has focused on health and ecosystem effects. With this workshop, our goal is to begin the discussion of risk management of suspected EDCs. To the best of my knowledge, this workshop is the first technical meeting ever conducted that focuses on risk management of suspected EDCs.

This workshop has been designed for stakeholders of environmental EDC problems, including:
- researchers from government, university and industrial labs who will develop risk management approaches
- Federal, State and local regulators who are charged with making risk management decisions
- engineering professionals who will implement risk management approaches
- industries associated with suspected EDCs who need to know the current state of the art in risk management of EDCs

Sooner or later many of you and your colleagues may be asked for input on managing the risk of EDCs. This workshop introduces you to the tools you will need:
- we begin with introductory talks about risk management - first in general then, how our colleagues in Europe are approaching risk management of EDCs
- next, we present speakers to bring you up to date on health effects and exposure assessment for suspected EDCs
- finally, we show the current status of applied risk management approaches for suspected EDCs.

Many people were involved in the planning and presentation of this workshop and in the development of this CD-ROM. I greatly appreciate all their hard work. Please see the credits page for more information.

We hope you find this multimedia, interactive CD-ROM a useful introduction to the current state of risk management of endocrine disrupting chemicals. We welcome your comments.

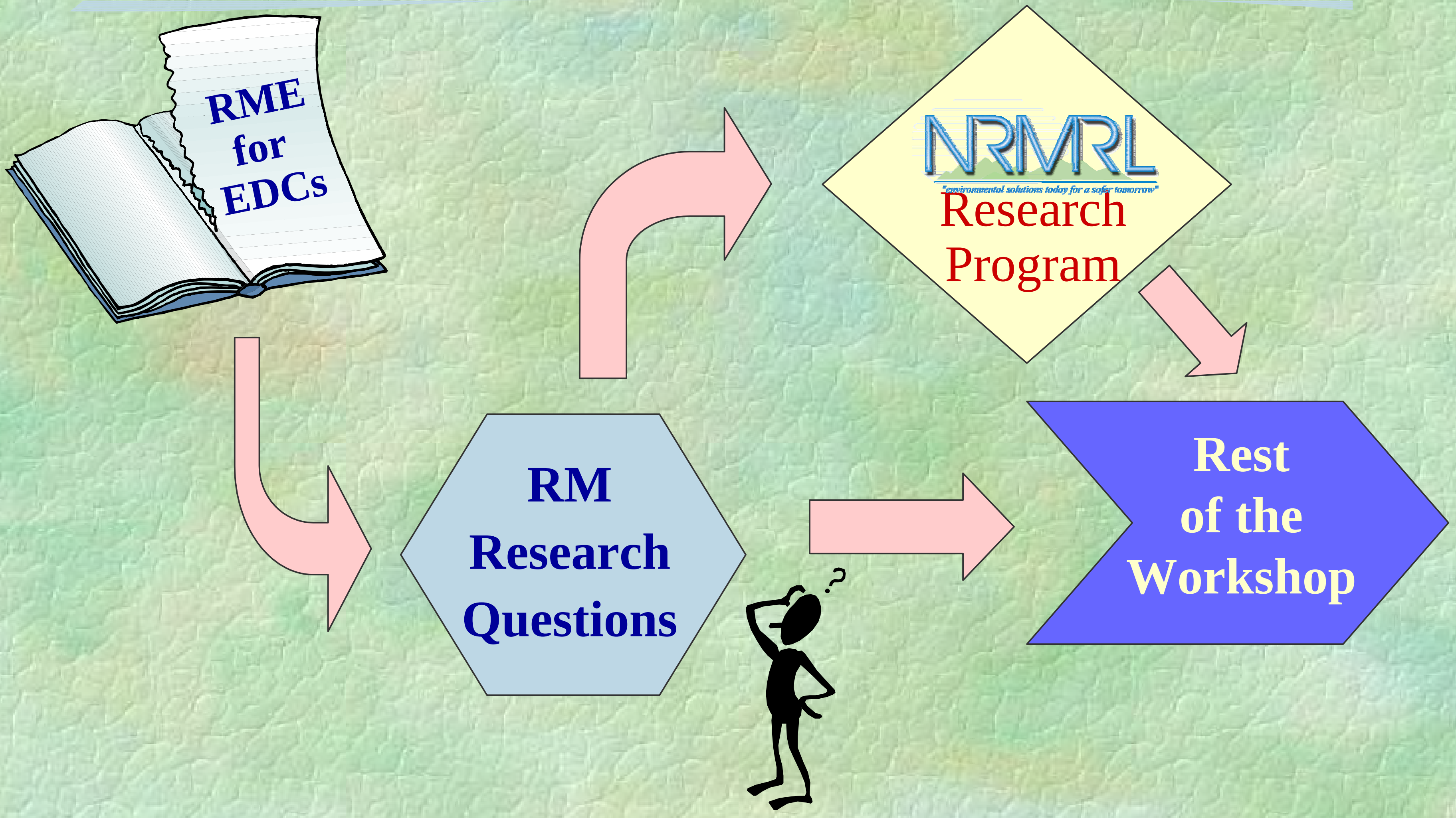
Thank you and enjoy.

EPA's Risk Management Evaluation of EDCs

Gregory D. Sayles
U.S EPA Office of Research and Development
National Risk Management Research Laboratory
Cincinnati, Ohio

"environmental solutions today for a safer tomorrow"

Presentation Overview



What is a Risk Management Evaluation (RME)?

The current understanding of risk management of an environmental challenge

Important sources of problem, environmental sinks

Currently available RM approaches

Identifies knowledge gaps, research needs

Uses for RMEs?

- Defines the environmental problem
- Gives current and future RM options to stakeholders (regulators, public interest groups, etc.)
- Allows environmental consultants / engineers to asses current skills and future investment
- Assists in research planning

Development of the RME Concept

- NRMRL developed several pilot RMEs for internal review
- One pilot RME was for EDCs
- Protocol for building RMEs drafted by NRMRL

The RME for Endocrine Disrupting Chemicals

<u>Writing Team</u>

Gregory Sayles, lead writer

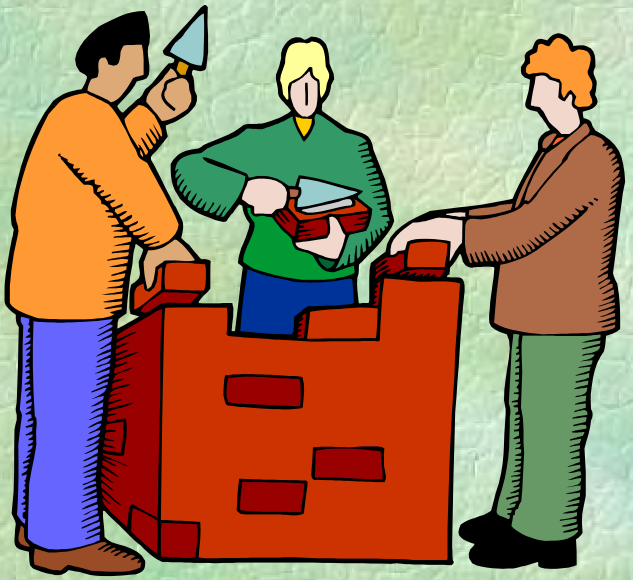
John Cicmanec

Steve Hutchins

Paul Lemieux

Carl Potter

Kathleen Schenck



Summary of Content

For a list of likely EDCs, describes

- Known health and eco effects
- Significant sources to the environment
- Significant exposures / environmental sinks
- Risk management tools
  - established
  - needed

RME Structure

- Chapter 1
- Chapter 2
- .
  .
- .
  .
- Chapter N-1
- Chapter N

RME Structure

- Chapter 1
- Chapter 2
- .
- .
- .
- .
- Chapter N-1
- Chapter N

- Intro to EDC problem
- Regulatory mandate
- Goals of document
- EDCs considered

RME Structure

- Chapter 1
- Chapter 2
- .
- .
- .
- .
- Chapter N-1
- Chapter N

- Known health, eco effects
- Known sources
- Known reservoirs
- Useful RM approaches
  - – established or new

RME Structure

- Chapter 1
- Chapter 2
- .
- .
- .
- .
- Chapter N-1
- Chapter N

Version 1.0

- Alkylphenols
- Steroid hormones: biogenic and pharmaceutical
- PCBs
- Chlorinated dioxins and furans
- DDT and DDE

RME Structure

- Chapter 1
- Chapter 2
- .
- .
- Chapter N-1
- Chapter N

Later versions

- Bisphenol A
- Phthalates
- Atrazine
- Methoxychlor
- Endosulfan
- Others?

RME Structure

- Chapter 1
- Chapter 2
- .
  .
- .
  .
- Chapter N-1
- Chapter N

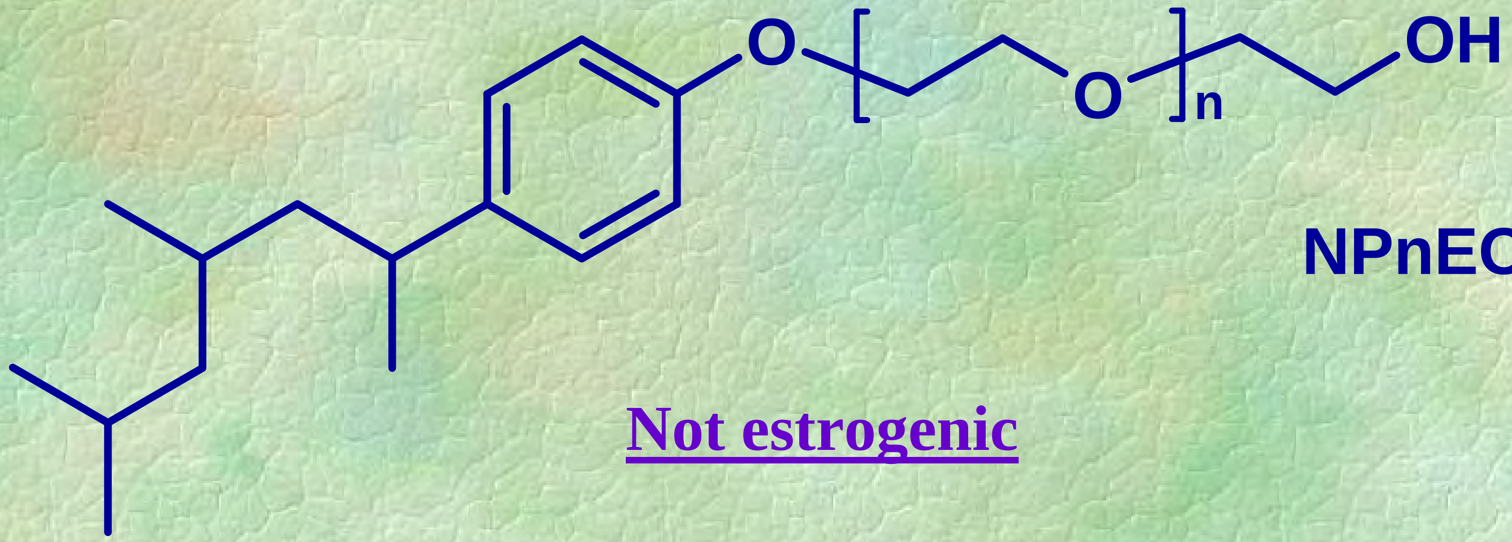
Summary of
- RM tools available
- RM research needs

Alkylphenolics, especially Nonylphenolics

- EDCs of interest:
  - Nonylphenol
  - Nonylphenol ethoxylates
  - Nonylphenol ethoxy carboxylic acids
- Eco effects – lab and field observation of estrogenic activity
- Human effects – none, except in vitro tests using human cell lines (estrogenic)

Parent Chemical

Nonylphenol polyethoxylate

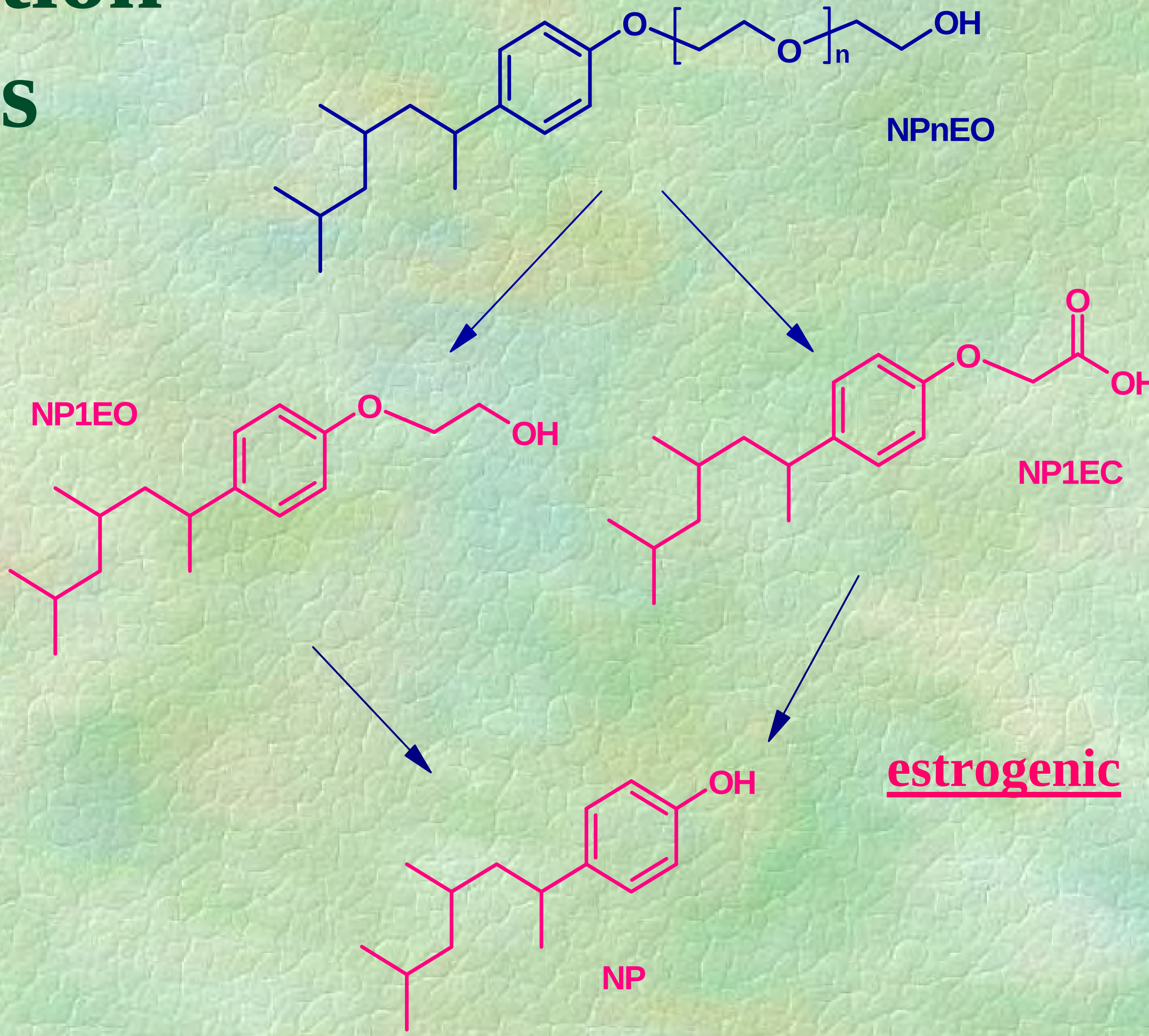


NPnEO

Not estrogenic

Generation of EDCs

More hydro-phobic



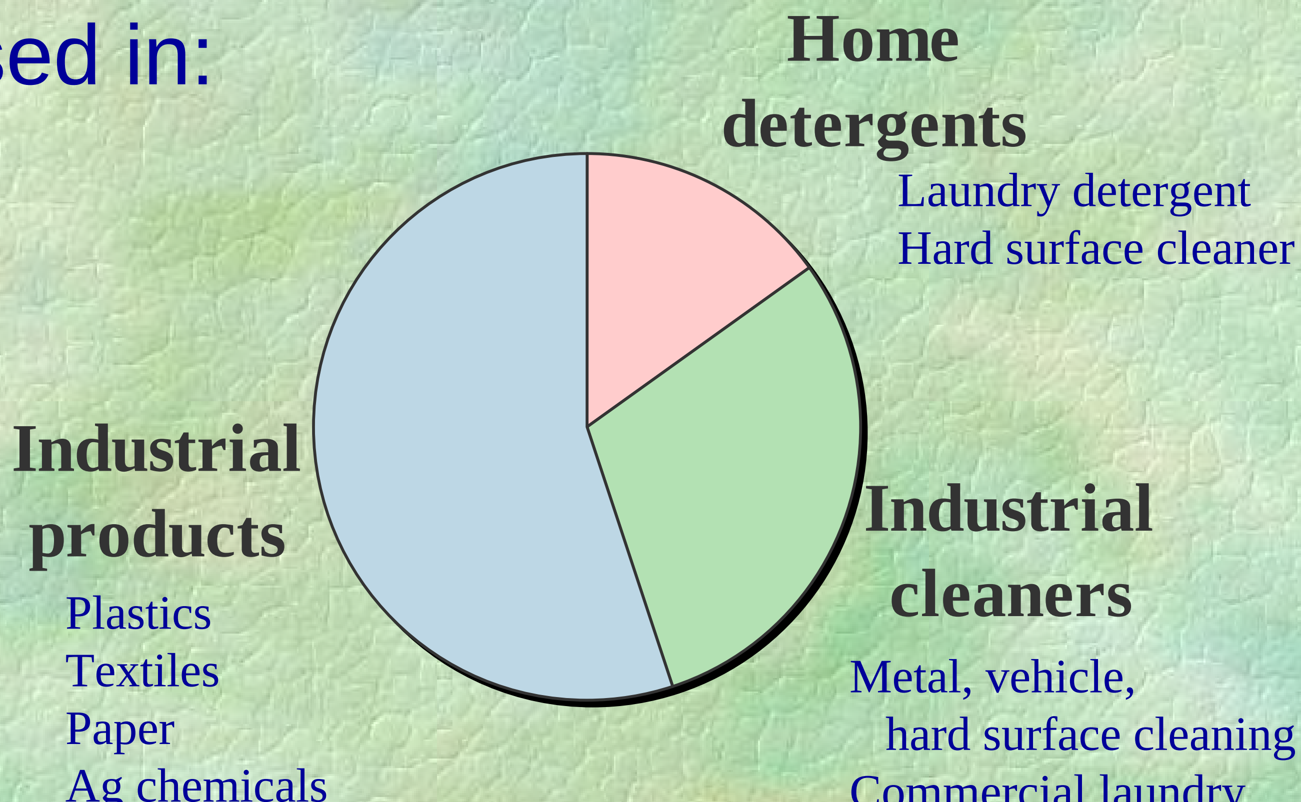
estrogenic

Uses for Nonylphenolics

- Nonylphenol
  - Antioxidant, lube oils
- Nonylphenol ethoxylate surfactants
  - Industrial and domestic detergents
  - Plastics, ag chemicals, paper production
  - Not EDCs
  - Biotransformed into EDCs in
    - sewage treatment plants
    - the environment

Market for NPE

- 350 - 500 million lbs / yr sold
- NPE used in:

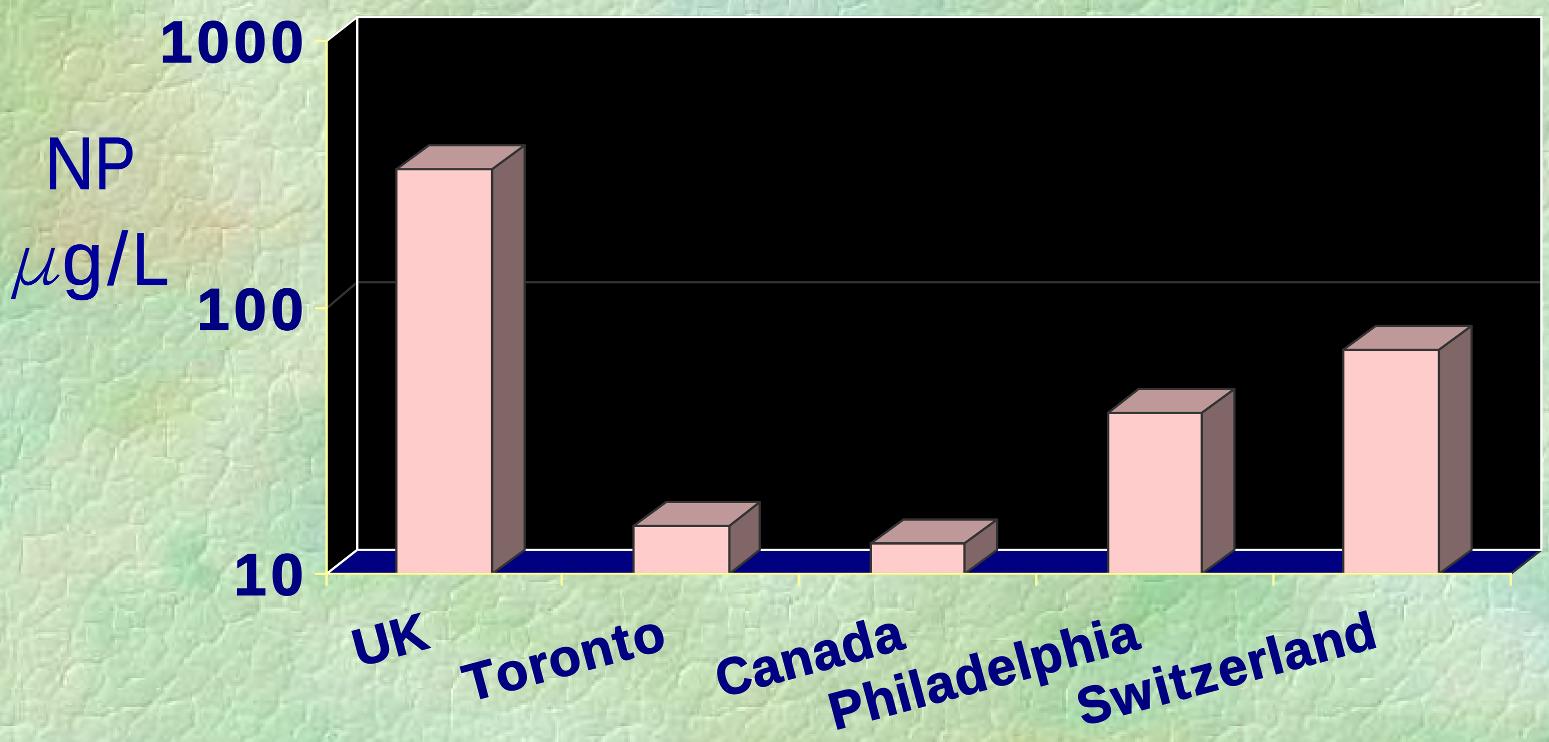


Likely Sources

- Wastewater treatment plants ✔
  - Domestic and industrial
- Pulp and paper mills ✔
- Pesticide use ✔
- Textile mills probably

Source Data

Sewage Treatment Effluent - Nonylphenol



Reservoir Data

Nonylphenol:

❖ Sediments - Canada	$\leq$ 44 mg/kg
❖ Surface Water – UK	$\leq$ 19 μg/L
❖ Groundwater - Cape Cod	30 μg/L
❖ Drinking water – Spain	$\leq$ 0.14 μg/L
❖ Air - NY City area	$\leq$ 25 ng/m^3

Risk Management Approaches

- Phase out / Product substitution
  - e.g., EU and alcohol ethoxylates
- Alter sewage treatment plant operation
  - Improve aqueous effluent
    - Add PAC to secondary treatment
    - Add tertiary GAC treatment
    - Add tertiary granular medium filtration
    - Alter secondary treatment process variables

Research Questions

- What is the performance of unit processes in STPs?
- Do current sludge disposal methods perform well?
- What is the capacity of aquatic sediments to manage the input of APs?

Research Questions

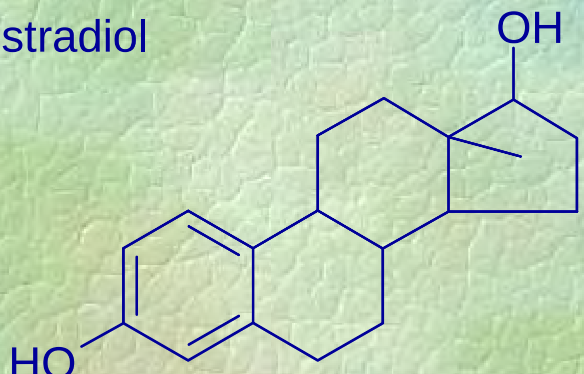
- Are other sewage treatment processes significant sources?
  - Septic systems
  - Constructed wetlands
- Are conventional drinking water treatment methods adequate? If not, are advanced treatment technologies?

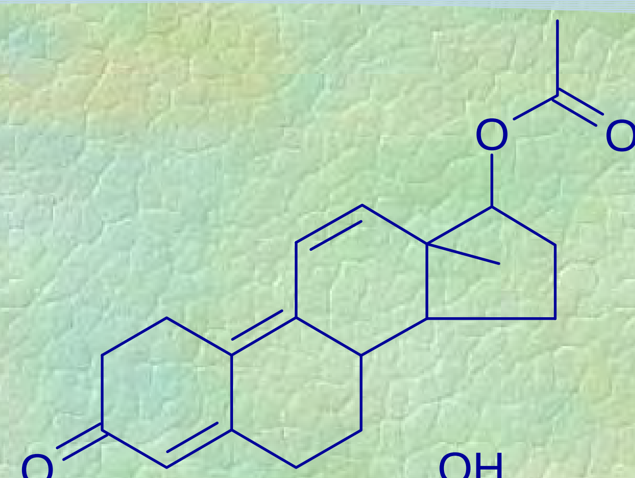
Biogenic / Pharmaceutical Steroid Hormones

- EDCs of interest:
  - natural steroid hormones, especially estrogens
  - Veterinary steroid hormones
- Effects – humans/mammals, birds, fish have steroid hormone receptors

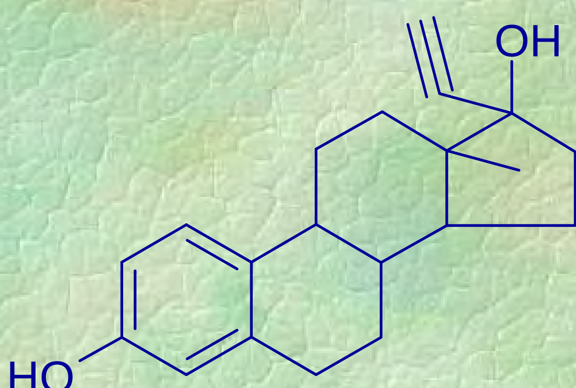
Structures of Some Hormones

estradiol

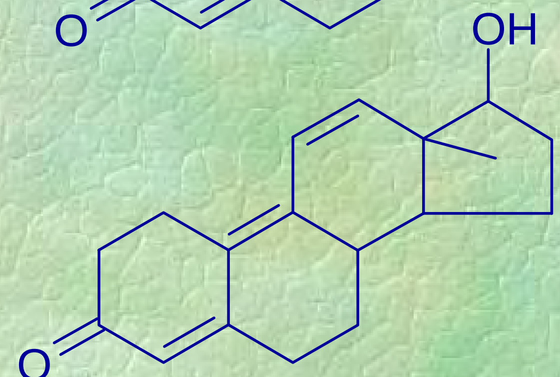




TbA



ethinyl estradiol



TbOH

Sources

- Sewage treatment plants

- Concentrated animal feeding operations (CAFOs)

Sinks / Reservoirs

- Surface water
  - Widely observed 1 – 10 ng/l
- Sediments
  - Expected accumulation - log K_{ow} = 3 - 4
- Ground water
  - Estradiol $\leq$ 60 ng/l (CAFO source)

RM Approaches

- Hormones aerobically biodegradable
- Will partition to activated carbon

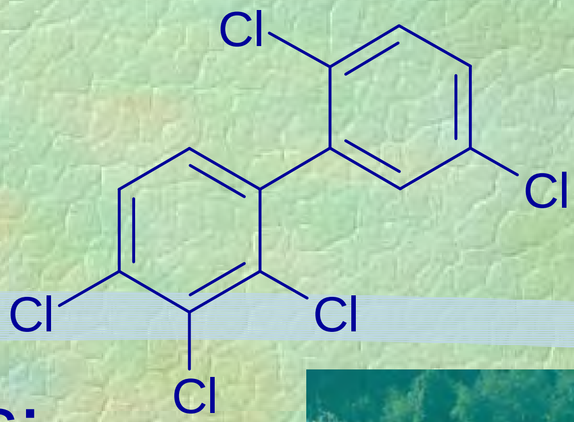
RM Research Questions

- What is the fate of steroid hormones in unit processes of STPs?
- How can STPs be improved to increase treatment?
- Are sludges significant reservoirs for steroids?
- Are sediments a significant sink? Are natural processes protective?

RM Research Questions

- What is the hormone content of animal waste?
- Do current waste management practices at CAFOs minimize environmental exposure? If not, how can they be improved?
- Does conventional drinking water treatment remove hormones? If not, are innovative approaches available?

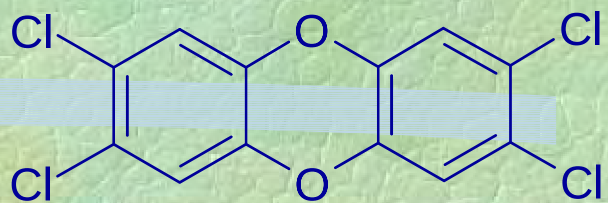
PCBs



❖ Research questions:

- What is the short term risk associated with dredging sediments?
- What is the long-term stability/effectiveness of capping sediments?
- When is natural attenuation/recovery of sediments effective?

Chlorinated Dioxins and Furans



- Research Questions
  - How can incinerators be operated to produce less chlorinated dioxins and furans
  - What combustion processes produce more/less endocrine active compounds?
  - How can the high producing processes be modified to minimize release?

RME - Evolution

- The *RME* is a living document
  - Version 1.0 will be completed Fall ‘02
  - Later versions roughly bi-annually
  - Peer reviewed

NRMRL's EDC Research Program

"environmental solutions today for a safer tomorrow"

- Project areas based on *RME* research questions
- EPA Principle Investigator driven

NRMRL's EDC Research Areas

"environmental solutions today for a safer tomorrow"

- Sewage Treatment
  - EDC fate within STPs
    - Paul McCauley / Dick Brenner
  - EDCs in sediments
    - Greg Sayles / Marc Mills

- Drinking water treatment
  - Effectiveness of conventional and advanced treatments
  - Kathy Schenck

NRMRL's EDC Research Areas

"environmental solutions today for a safer tomorrow"

- Confined Animal Feeding Operations
  - CAFOs as a source of EDCs
  - Effectiveness of waste management practices
  - Steve Hutchins / Carl Enfield
- Pollution Prevention
  - Tool development for identification of EDC substitutes
  - Doug Young

NRMRL's EDC Research Areas

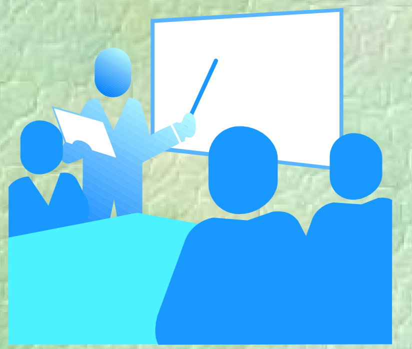
"environmental solutions today for a safer tomorrow"

- Combustion Characterization
  - EDC content of various process effluents
  - Brian Gullett
- Bioassays for RM Performance Evaluation
  - Bioassays show big picture performance of RM
  - Carolyn Acheson

NRMRL's EDC Research Areas

"environmental solutions today for a safer tomorrow"

- Technical Information Transfer
  - Summary documents, web site, workshops

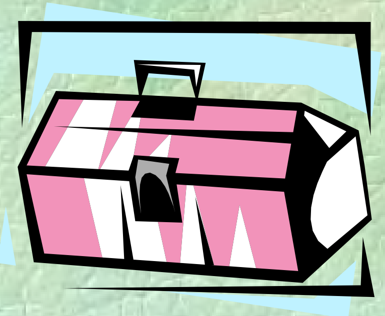


RM Decision-Making Tools

- Risk management decisions for EDCs will be required more frequently
- What RM tools do you have?
  - This *Workshop* (in person, CD-ROM)
  - *Risk Management Evaluation of EDCs*
  - EPA's RM of EDCs web page www.epa.gov/NRMRL/EDC
- What RM tools are being developed?
  - EPA's RM research program
  - Others' research

Summary

- *Risk Management Evaluation of EDCs*
  - Will help you follow state of the art in RM
  - Provide input to research agendas
- RME drives NRMRL research
- Build your tool box



Biological Fate of Estrogenic Compounds Associated with Sewage Treatment: A Review

Gregory Sayles
U.S. EPA

Tamara Marsh
Elmhurst College, IL

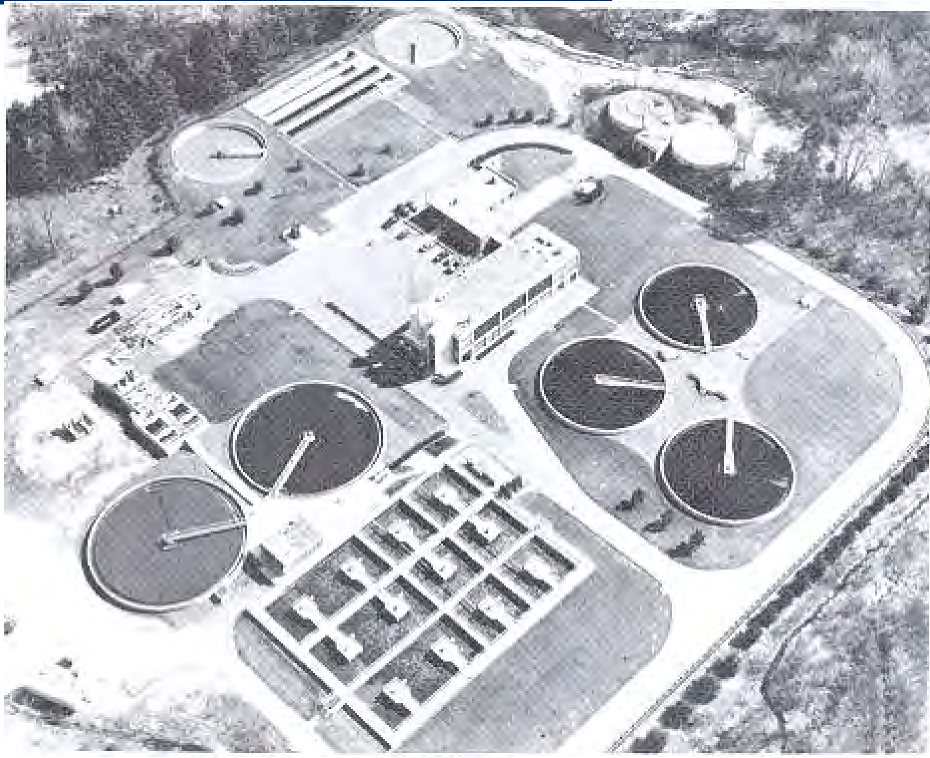
EPA

NRMRL
"environmental solutions today for a safer tomorrow"

Elmhurst College

Purpose

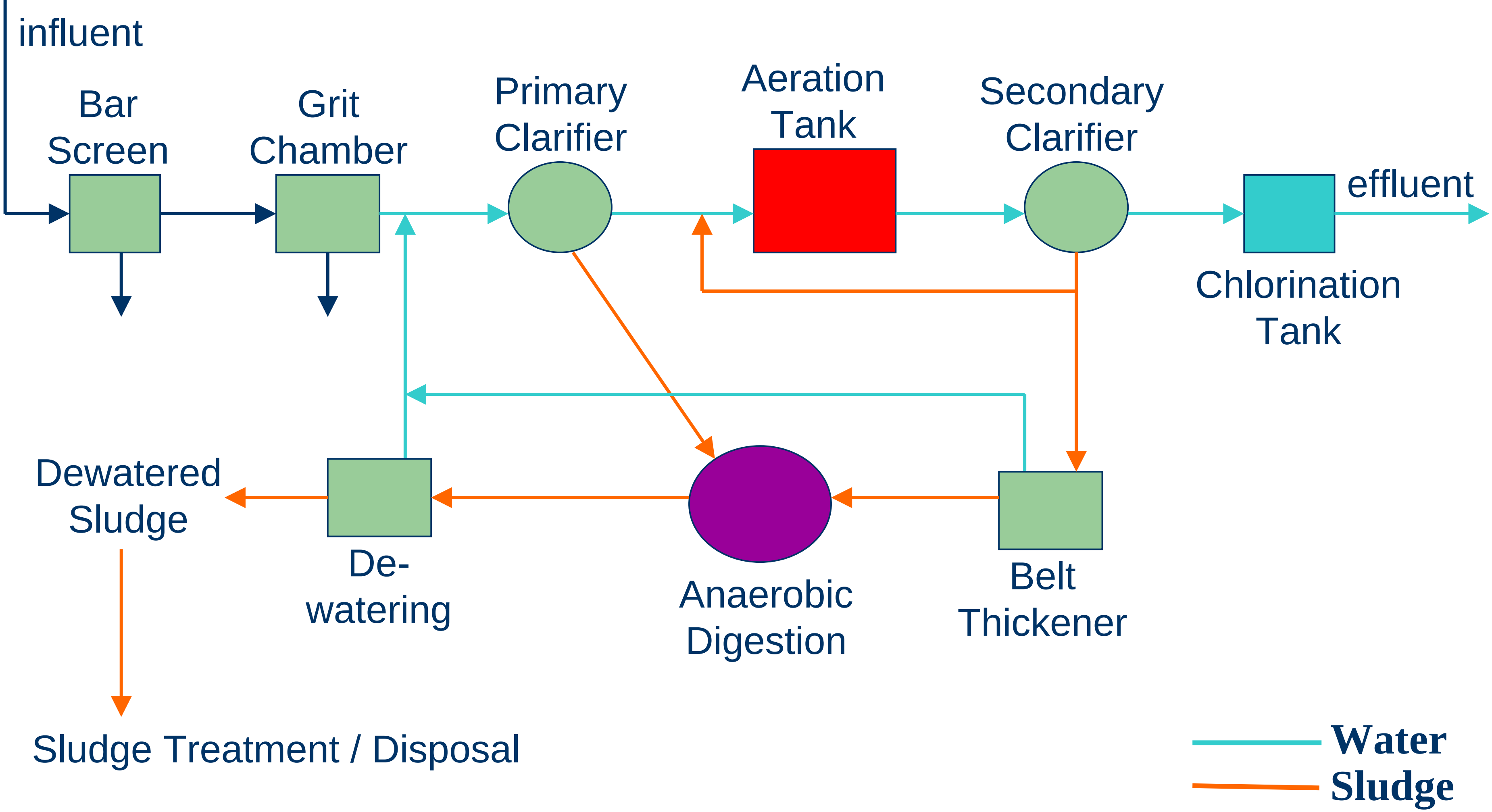
- Give you current thinking on fate of EDCs in sewage treatment systems
  - Available data
  - Qualitative discussion



Most Prevalent Estrogenic Chemicals found in STP Effluent

- Alkylphenolic compounds
  - Biodegradation products from APE surfactants
    - Nonylphenolics
    - Octylphenolics
- Steroid hormones
  - Natural (estradiol, estrone, estriol)
  - Synthetic (ethinyl estradiol)

Typical Large STP



STPs Designed for…

- BOD removal
- Suspended solids removal
- pH neutralization
- N, P removal
- Pathogen removal

Current Knowledge Limited

- For nonylphenolics:
  - Parent NPE are a distribution of EO units
  - > 20 isomers of the nonyl group
  - Analytical standards for NPE metabolites difficult to obtain
- For steroids and nonylphenolics:
  - Analytical methods not standardized
  - Low detection limits needed (ng/l)
- Few biodegradation studies published

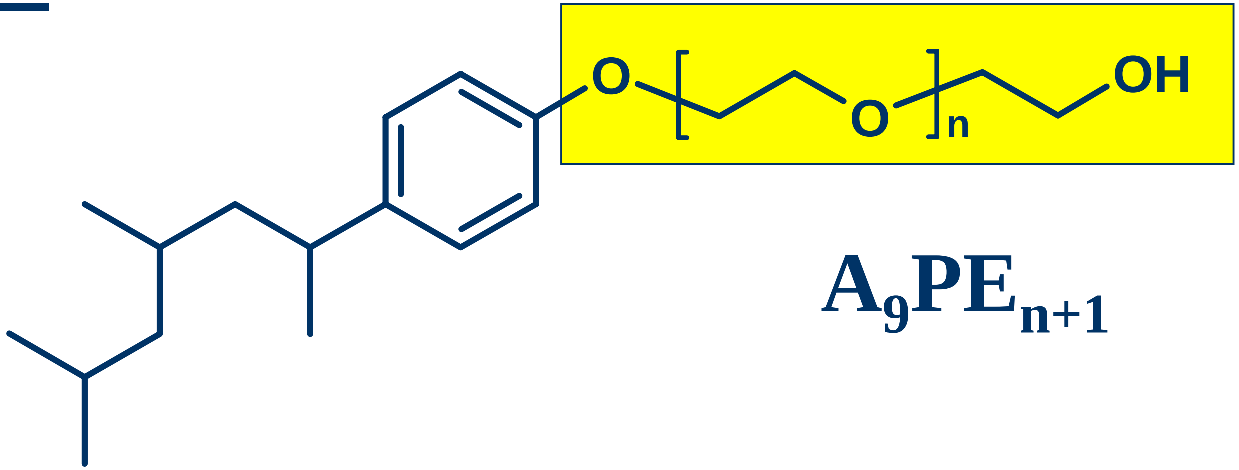
NPEs - STP Influent / Raw Sewage

- ~ 500 million pounds used per year in U.S.
- Institutional: laundry detergents, janitorial and vehicle cleaners
- Household: cleaners and personal care products
- Industrial: plastics, textiles and pulp and paper processing, ag chemicals

Total nonylphenolics	500 – 2500 ug/l
Total estrogenic products	1 – 50 ug/l

Biological Transformation of APEs

Aerobic

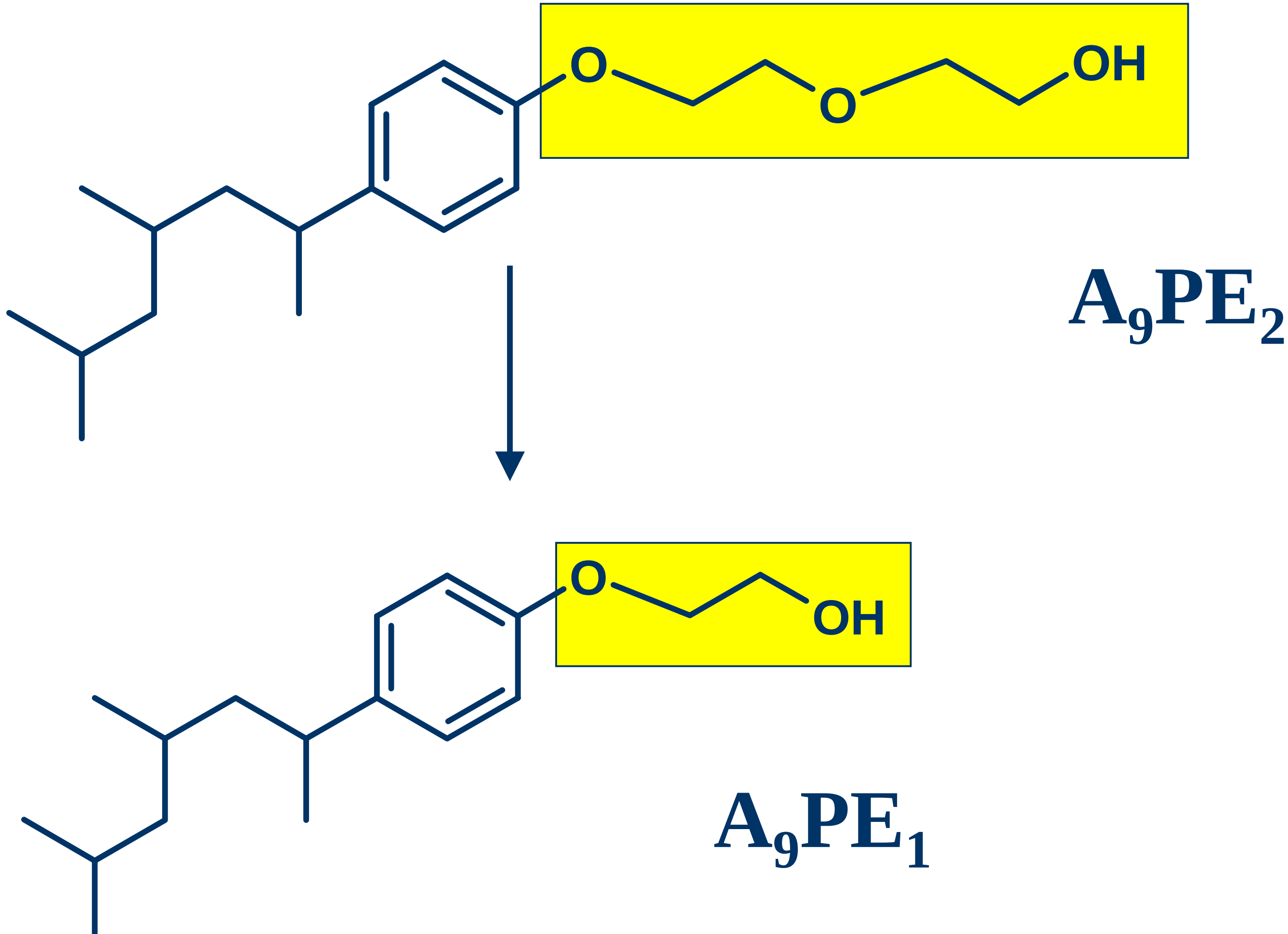


A_9PE_{n+1}

Parent surfactant
"nonylphenol polyethoxylate"

Biological Transformation of APEs

Aerobic

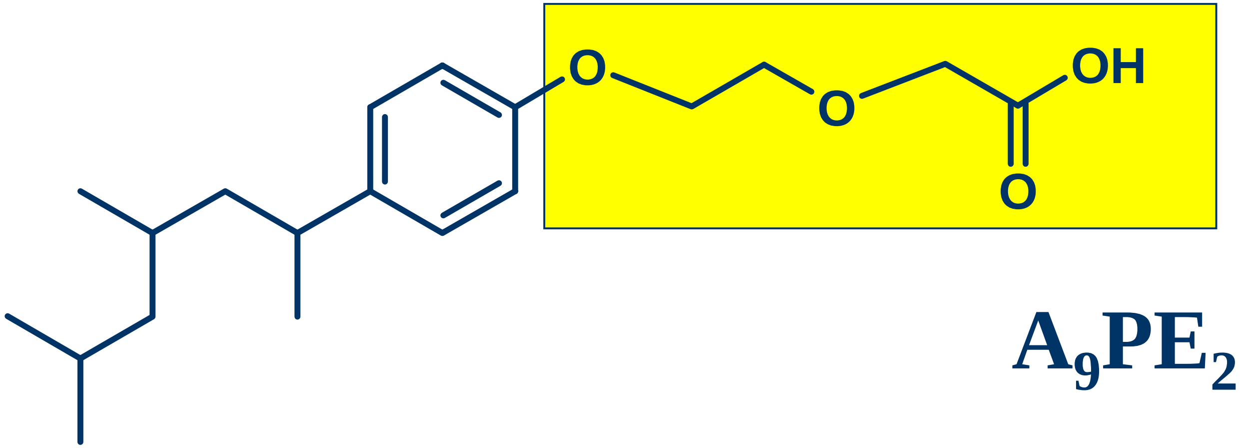


A_9PE_2

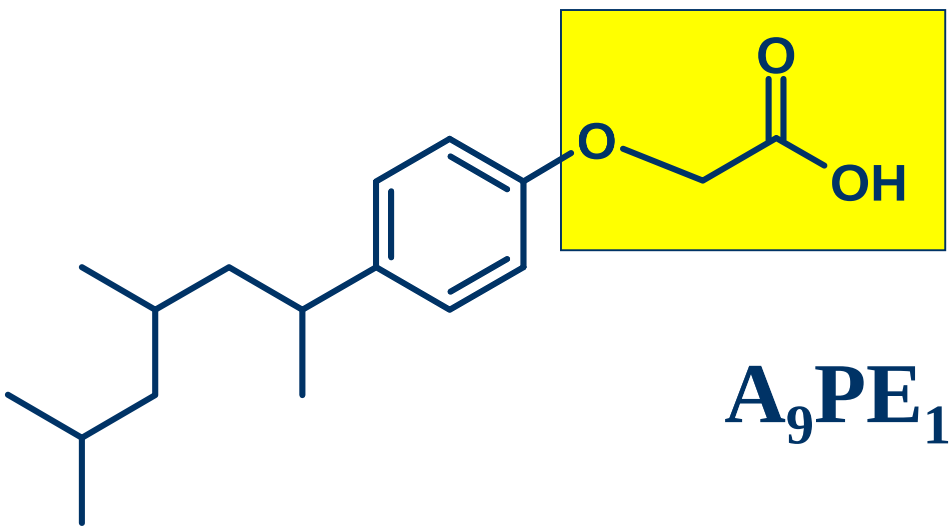
A_9PE_1

Biological Transformation of APEs

Aerobic



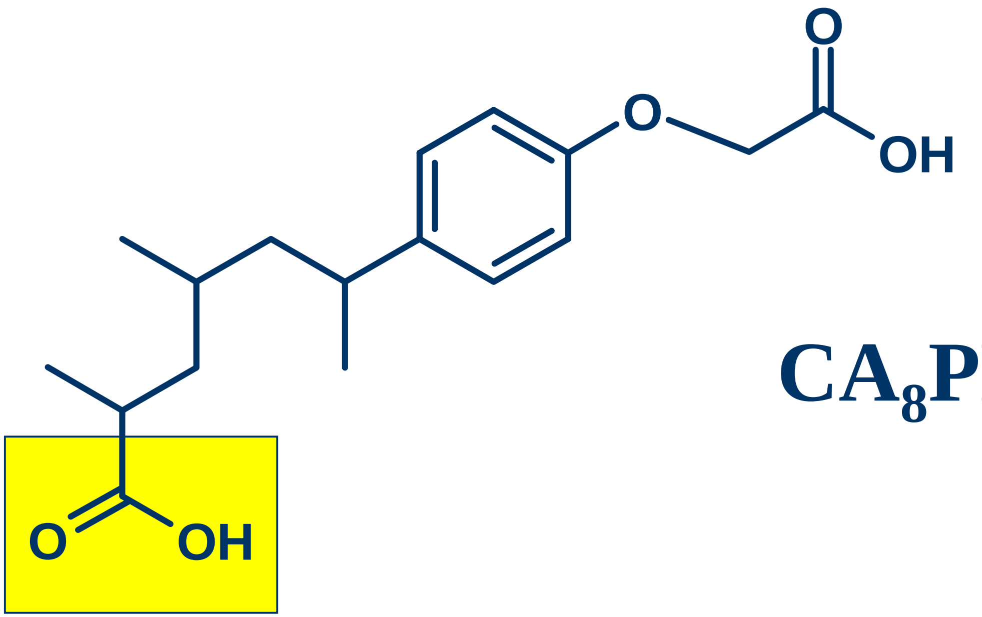
A_9PE_2C



A_9PE_1C

Biological Transformation of APEs

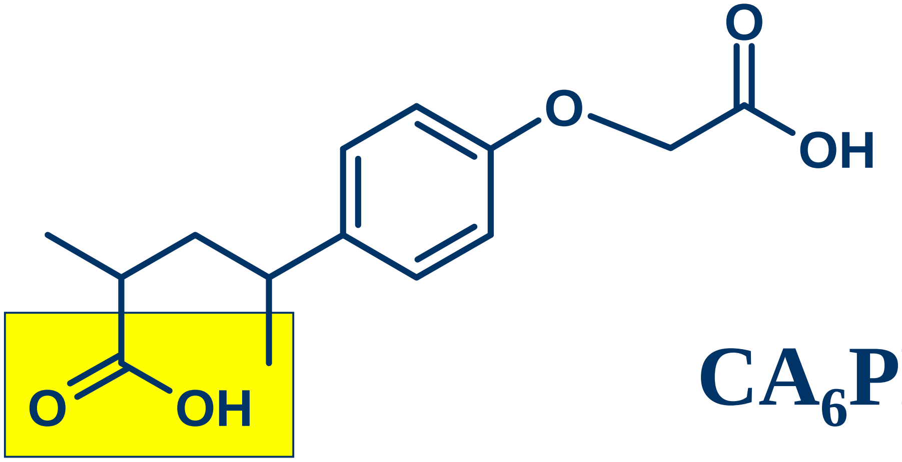
Aerobic



CA_8PE_1C

Biological Transformation of APEs

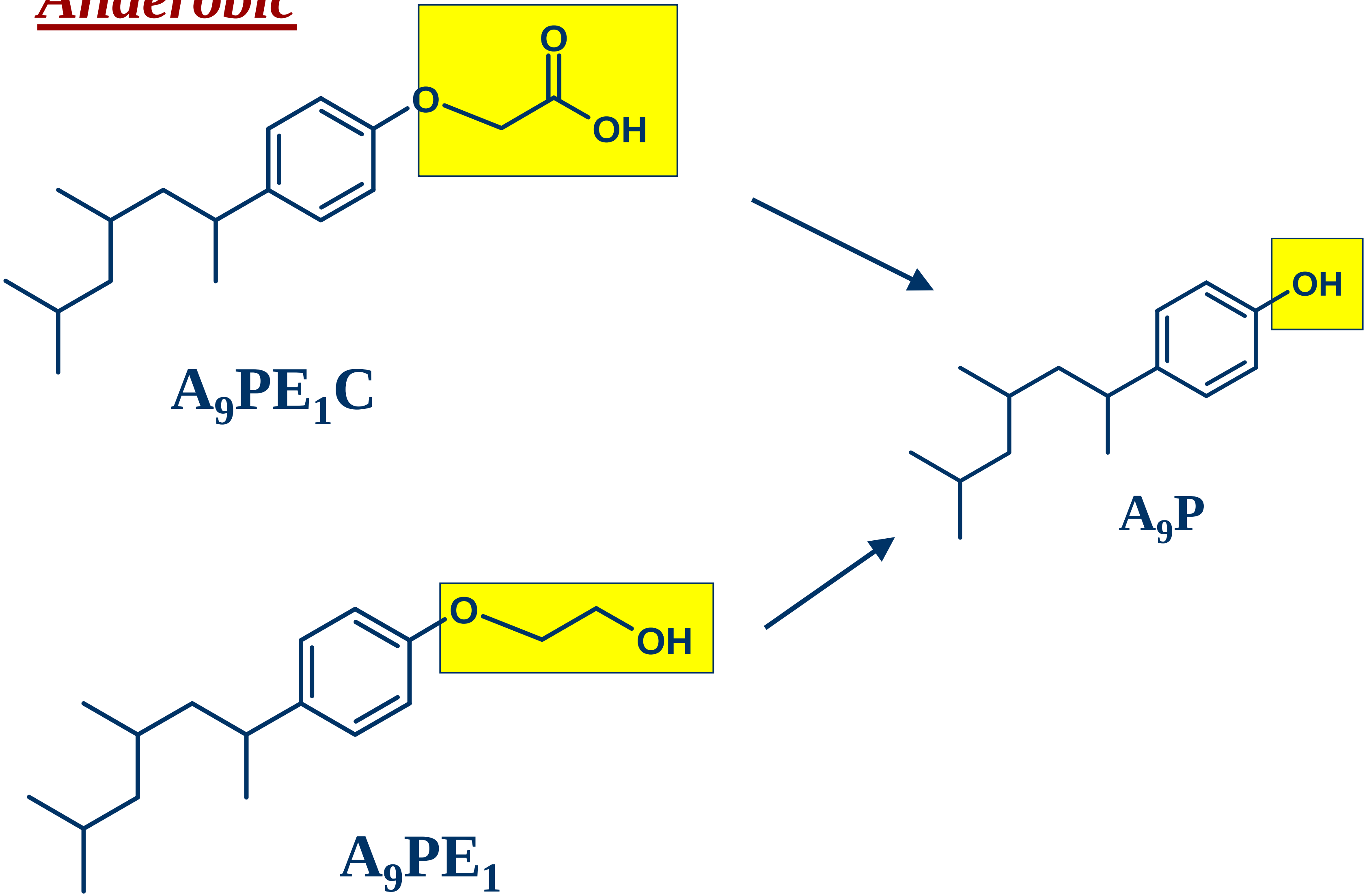
Aerobic



CA_6PE_1C

Biological Transformation of APEs

Anaerobic

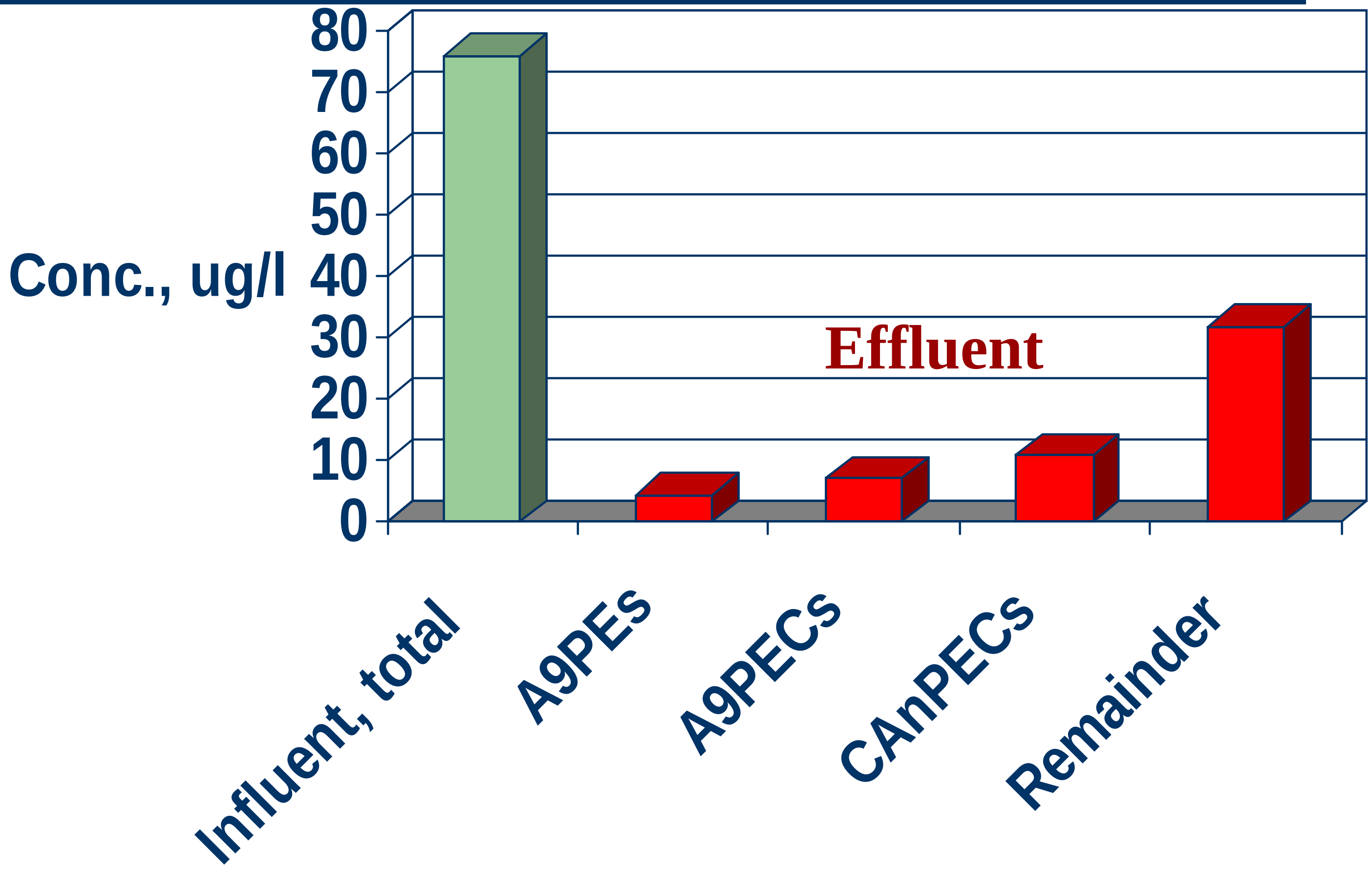


A_9PE_1C

A_9P

A_9PE_1

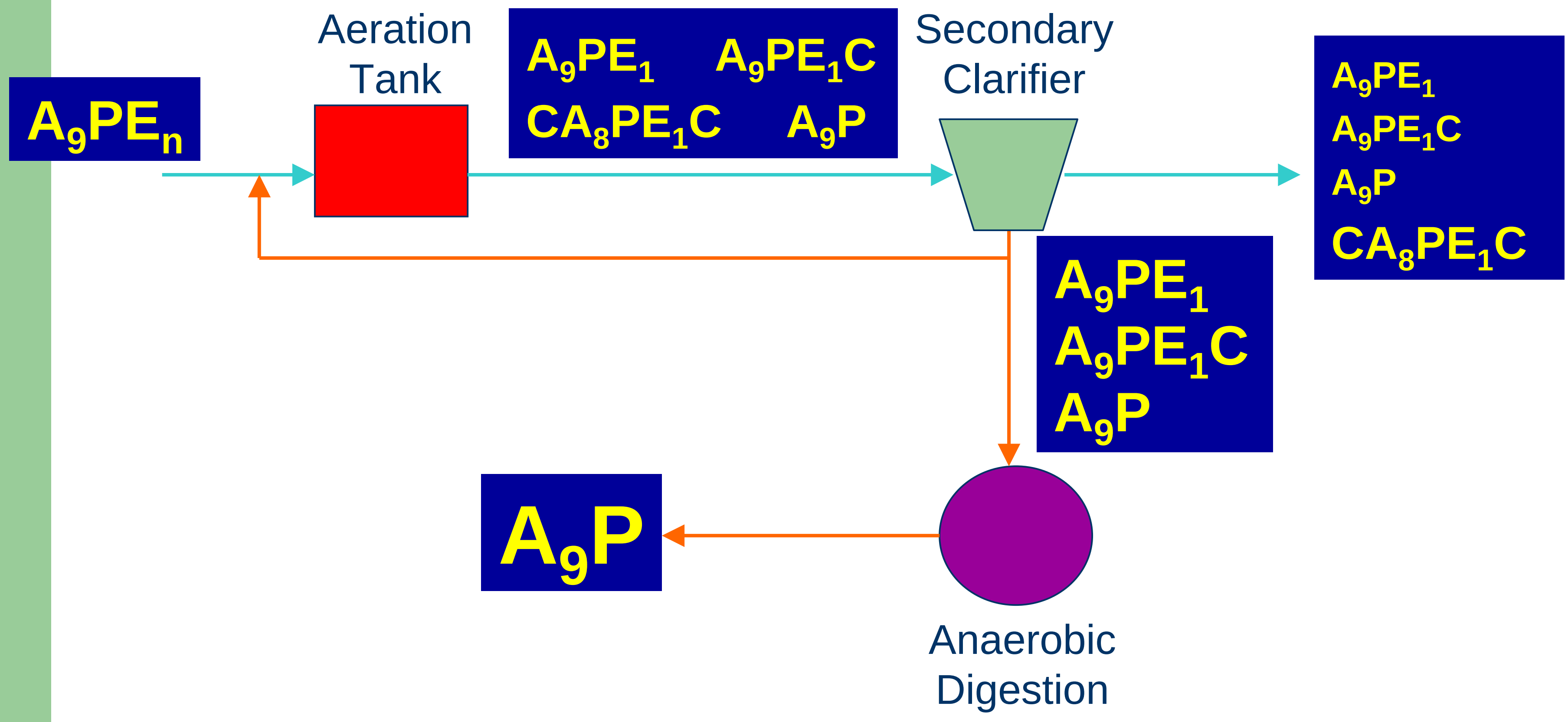
Nonylphenolic STP Balance Rome, Italy (Di Corcia, 2000)



NPE Metabolites Partition to Sludge

EDC	log (Kow)
NP	4.5
NP1EO	4.2
NP2EO	4.2
CNP1EC	<< 4

Likely Fate in STP

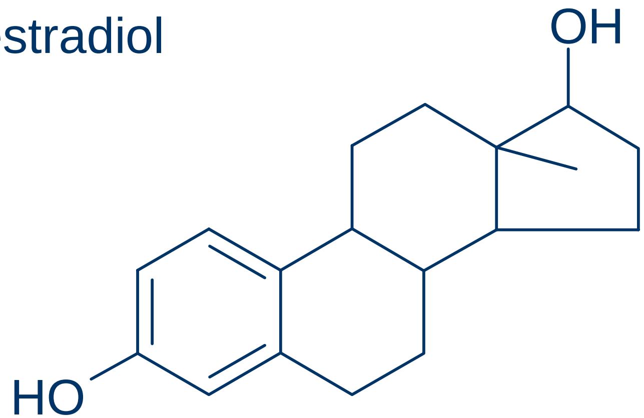


Effluent Fate

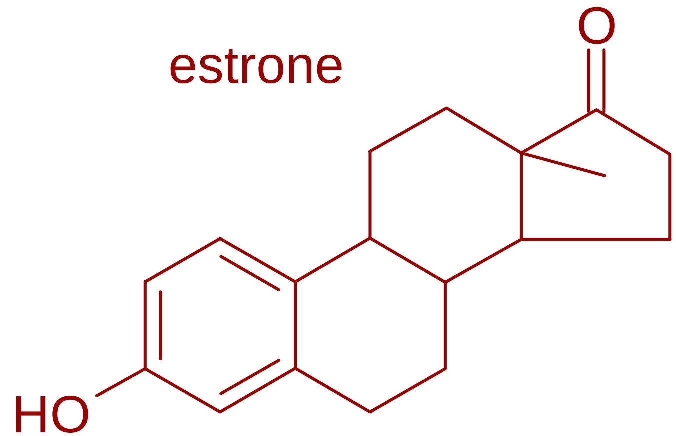
- Expect alkyphenolics in
  - Surface water (up to 20 ug/l)
  - Sludges (up to 50 mg/kg)
  - Sediments (up to 4000 mg/kg)

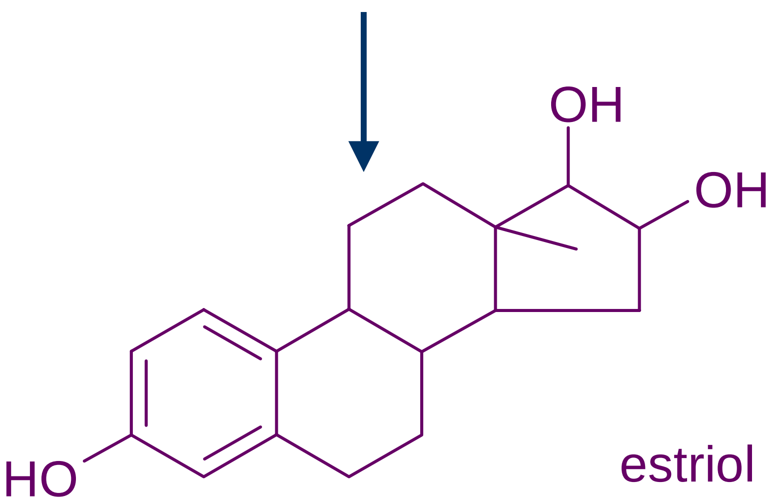
Influent Estrogens

estradiol

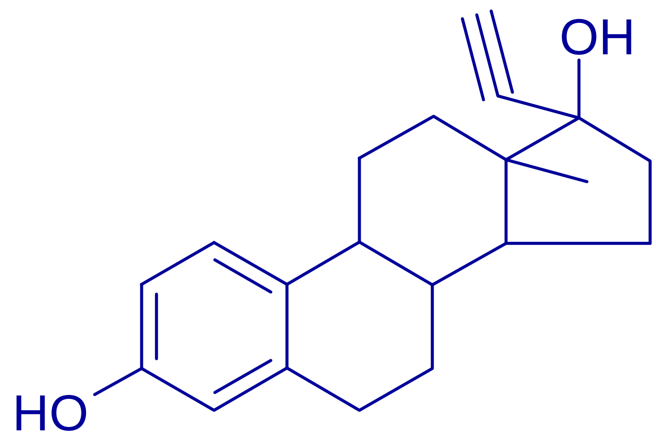


estrone



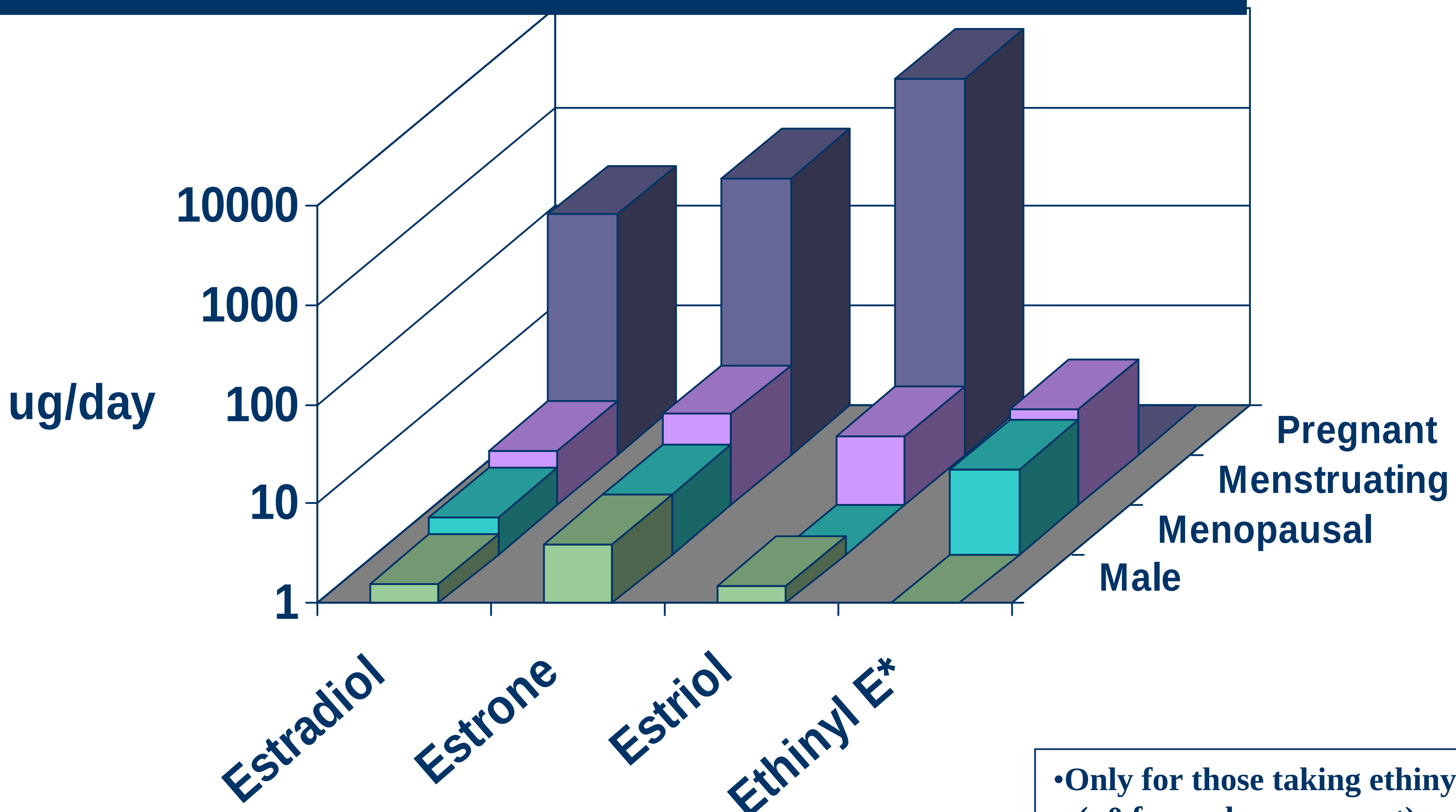


estriol



ethinyl estradiol

Amount of Estrogens Excreted



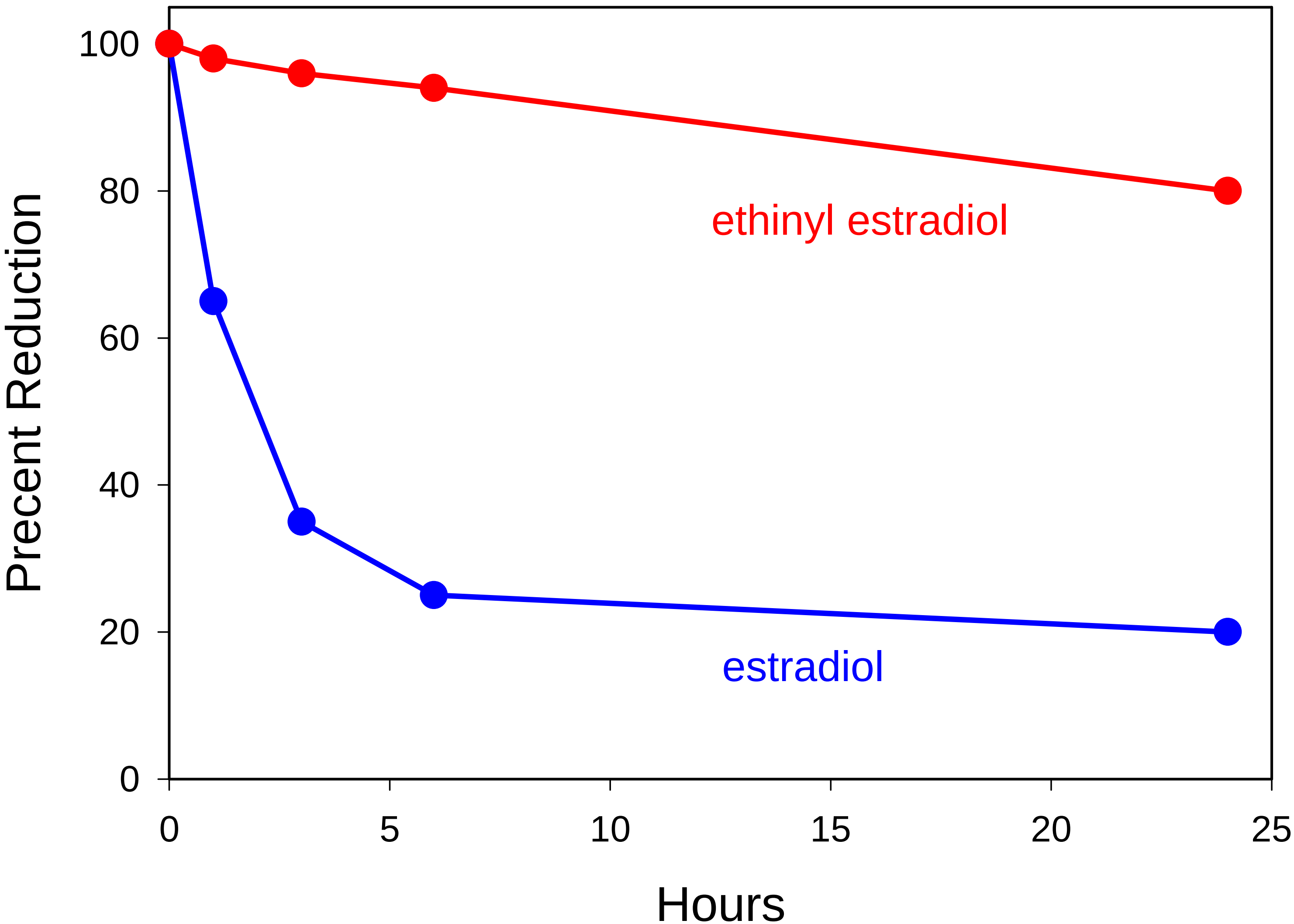
•Only for those taking ethinyl (=0 for males, pregnant)

STP Influent - Raw Sewage

❖ Estradiol	ND – 50 ng/l
❖ Estriol, estrone	ND – 100 ng/l
❖ Ethinyl estradiol	ND – 10 ng/l

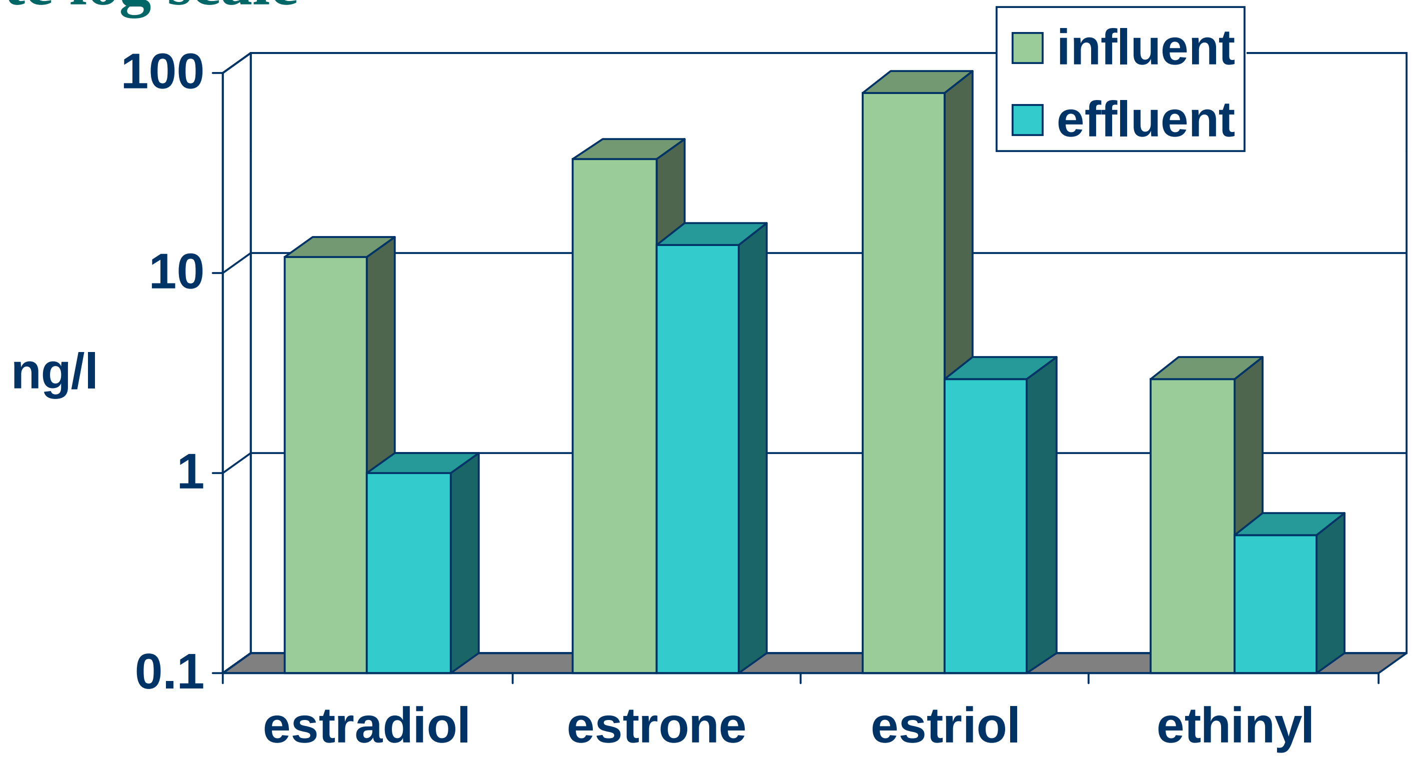
Estrogens in Activated Sludge

From 6 STPs in Tennessee (Layton, 2000)



Example STP Removal – Rome Italy

Note log scale



See Baronti, 2000

Biodegradation of Estrogens

- Aerobic
  - All biodegradable, ethinyl slowest
  - Nitrification may increase rate
- Anaerobic
  - Little known
  - Little biodegradation expected

Partition to Sludge, Sediments?

EDC	log (Kow)
Ethinyl estradiol	4.2
Estradiol	3.9
Estrone	3.4
Estriol	2.8

No sludge, sediments field data to date

Overall Impact of Various EDCs in STPs (Johnson, 2001)

Chemical	Estradiol Equiv. (in vivo studies)	Level of Concern
Ethinyl estradiol	12	High
Nonyl-, octyl-phenol	2 – 20	Moderate
Estrone	2.5	Moderate
Estradiol	1.0	Moderate
Estriol	0.02	Lowest
APEs, APECs, CAPEs	?	Low?

Summary

- Current limited data
  - Aerobic biodegradation produces estrogenic alkylphenolics in STPs
  - No evidence of destruction of alkylphenolics in STPs
  - Steroid hormones are aerobically biodegradable
    - Retention time in STP appears to be short
  - Sludge will accumulate low MW alkylphenolics and hormones
- Need detailed studies of EDC fate within the STP
- Are other sewage treatment systems removing EDCs?
  - Septic systems?
  - Small community treatment, constructed wetlands?

Bibliography

Arcand-Hoy, L. D., A. C. Nimrod and W. H. Benson (1998). "Endocrine-Modulating Substances In The Environment: Estrogenic Effects of Pharmaceutical Products." International Journal of Toxicology **17**: 139-158.

Baronti, C., R. Curini, G. D'Ascenzo, A. Di Corcia, A. Gentili and R. Samperi (2000). "Monitoring Natural and Synthetic Estrogens at Activated Sludge Sewage Treatment Plants and in a Receiving River Water." Environmental Science & Technology **34**(24): 5059-5065.

Belfroid, A. C., A. Van der Horst, A. D. Vethaak, A. J. Schafer, G. B. J. Rijs, J. Wegener and W. P. Cofino (1999). "Analysis and Occurence of Estrogenic Hormones and Their Glucuronides in Surface Water and Waste Water in The Netherlands." The Science of the Total Environment **225**: 101-108.

Benne, D. T. (1999). "Review of the Environmental Occurence of Alkylphenols and Alkylphenol Ethoxylates." Water Quality Research Journal of Canada **34**(1): 79-122.

Bolz, U., H. Hagenmaier and W. Korner (2001). "Phenolic Xenoestrogens in Surface Water, Sediments, and Sewage Sludge from Baden-Wurttemberg, South-West Germany." Environmental Pollution **115**: 291-301.

Desbrow, C., E. J. Routledge, G. C. Brighty, J. P. Sumpter and M. Waldock (1998). "Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical Fractionation and in Vitro Biological Screening." Environmental Science & Technology **32**(11): 1549-1558.

Di Corcia, A., R. Cavallo, C. Crescenzi and M. Nazzari (2000). "Occurrence and Abundance of Dicarboxylated Metabolites of Nonylphenol Polyethoxylate Surfactants in Treated Sewages." Environmental Science & Technology **34**(18).

Fawell, J. K., D. Sheahan, H. A. James, M. Hurst and S. Scott (2001). "Oestrogens and Oestrogenic Activity in Raw and Treated Water in Severn Trent Water." Water Research **35**(5): 1240-1244.

Ferguson, P. L., C. R. Iden and B. J. Brownawell (2001). "Distribution and Fate of Neutral Alkylphenol Ethoxylate Metabolites in a Sewage-Impacted Urban Estuary." Environmental Science Technology **35**(12): 2428-2435.

Bibliography

Furbacker, M., A. Breithofer and A. Junghauer (1999). "17beta-Estradiol: Behavior During Waste Water Analyses." Chemosphere **39**(11): 1903-1909.

Giger, W., M. Ahel, M. Koch, H. U. Laubscher, C. Schaffner and J. Schneider (1987). "Behaviour of Alkylphenol Polyethoxylate Surfactants and of Nitrilotriacetate in Sewage Treatment." Water Science and Technology **19**: 449-460.

Giger, W., P. H. Brunner and C. Schaffner (1984). "Nonylphenol in Sewage Sludge: Accumulation of Toxic Metabolites from Nonionic Surfactants." Science **225**: 623-625.

Hale, R. C., C. L. Smith, P. O. de Fur, E. Harvey, E. O. Bush, M. J. La Guardia and G. C. Vadas (2000). "Nonylphenols in Sediments and Effluents Associated with Diverse Wastewater Outfalls." Environmental Toxicology and Chemistry **19**(4): 946-952.

Hesselsoe, M., D. Jensen, K. Skals, T. Olesen, P. Moldrup, P. Roslev, G. K. Mortensen and K. Henriksen "Degradation of 4-Nonylphenol in Homogeneous and Nonhomogeneous Mixtures of Soil and Sewage Sludge." Environmental Science & Technology.

Johnson, A. C., A. Belfroid and A. Di Corcia (2000). "Estimating Steroid Oestrogen Inputs Into Activated Sludge Treatment Works and Observations on Their Removal From the Effluent." The Science of the Total Environment **256**: 163-173.

Johnson, A. C., R. J. Williams and T. Ulahannan (1999). "Comment on "Identification of Estrogenic Chemicals in STW Effluent..1. Chemical Fractionation and in Vitro Biological Screening"." Environmental Science Technology **33**(2): 369-370.

Korner, W., U. Bolz, W. Susmuth, G. Hiller, W. Schuller, V. Hanf and H. Hagenmaier (2000). "Input/Output Balance of Estrogenic Acitve Compounds in a Major Municpal Sewage Plant in Germany." Chemosphere **40**: 1131-1142.

La Guardia, M. J., R. C. Hale, E. Harvey and T. M. Mainor (2001). "Alkylphenol Ethoxylate Degradation Products in Land-Applied Sewage Sludge (Biosolids)." Environmental Science & Technology **35**(24): 4798-4804.

MaGuire, R. J. (1999). "Review of the Persistence of Nonylphenol and Nonylphenol Ethoxylates in Aquatic Environments." Water Quality Journal of Canada **34**(1): 37-78.

Marcomini, A., F. Cecchi and A. Sfriso (1991). "Analytical Extraction and Environmental Removal of Alkylbenzene Sulphonates, Nonylphenol and Nonylphenol Monoethoxlate from Dated Sludge-Only Landfills." Environmental Technology **12**: 1047-1084.

Bibliography

Routledge, E. J., D. Sheahan, C. Desbrow, G. C. Brighty, M. Waldock and J. P. Sumpter (1998). "Identification of Estrogenic Chemicals in STW Effluent. 2. In Vivo Responses in Trout and Roach." Environmental Science & Technology **32**(11): 1559-1565.

Sekela, M., R. Brewer, G. Moyle and T. Tuominen (1999). "Occurence of An Environmental Estrogen (4-Nonylphenol) in Sewage Treatment Plant Effluent and the Aquatic Receiving Environment." Water Science and Technology **39**(10-11): 217-220.

Shishida, K., S. Echigo, K. Kosaka, M. Tabaski, T. Matsuda, H. Takigami, H. Yamada, Y. Shimizu and S. Matsui (2000). "Evaluation of Advanced Sewage Treatment Processes for Reuse of Wastewater Using Bioassays." Environmental Technology **21**: 553-560.

Ternes, T. A., P. Kreckel and J. Mueller (1999). "Behavior and Occurrence of Estrogens in Municipal Sewage Treatment Plants - II. Aerobic Batch Experiments with Activiated Sludge." The Science of the Total Environment **225**: 91-99.

Ternes, T. A., M. Stumpf, J. Mueller, K. Haberer, R.-D. Wilken and M. Servos (1999). "Behavior and Occurrence of Estrogens in Municipal Sewage Treatment Plants - I. Investigations in Germany, Canada and Brazil." The Science of the Total Environment **225**: 81-90.

Williams, R. J., M. D. Jurgens and A. C. Johnson (1999). "Initial Predictions of the Concentrations and Distribution of 17beta-Oestradiol, Oestrone and Ethinyl Oestradiol in 3 English Rivers." Water Research **33**(No. 7): 1663-1671.

Evaluation of Drinking Water Treatment Technologies for Removal of Endocrine Disrupting Compounds

Kathleen Schenck and Thomas Speth, U.S. EPA, NRMRL
Laura Rosenblum, Steve Wendelken, Barry Pepich, and Radha Krishnan, IT Corporation
Kent Mitchell and David Warshawsky, University of Cincinnati

Many of the chemicals identified as potential endocrine disrupting compounds (EDCs) may be present in surface or ground waters used as drinking water sources due to their introduction from:

- Domestic and industrial sewage treatment systems.
- Wet-weather runoff.

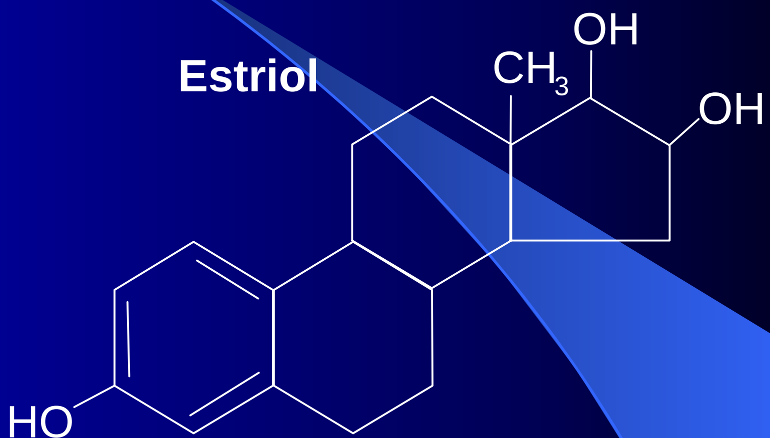
Basic strategies to decrease the potential risk of adverse health effects associated with the presence of EDCs in drinking water:

- Protect drinking water sources from contamination by EDCs.
- Remove EDCs, that may be present in source waters, during drinking water treatment.

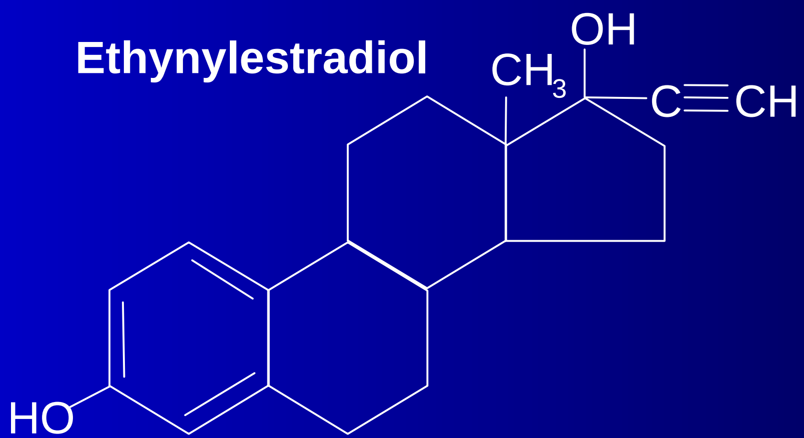
Compounds to be evaluated

Estradiol

Estriol

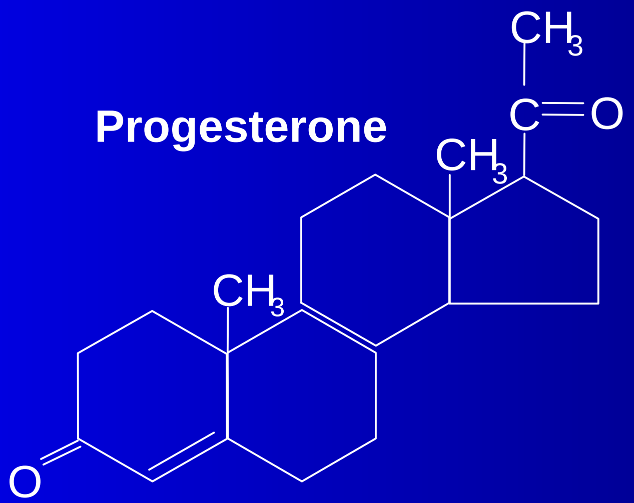


Ethynylestradiol

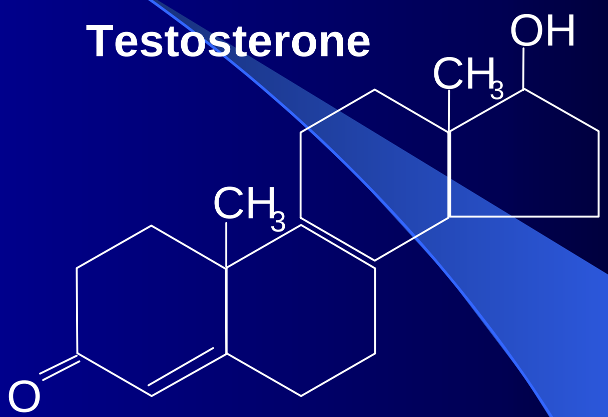


Compounds to be evaluated

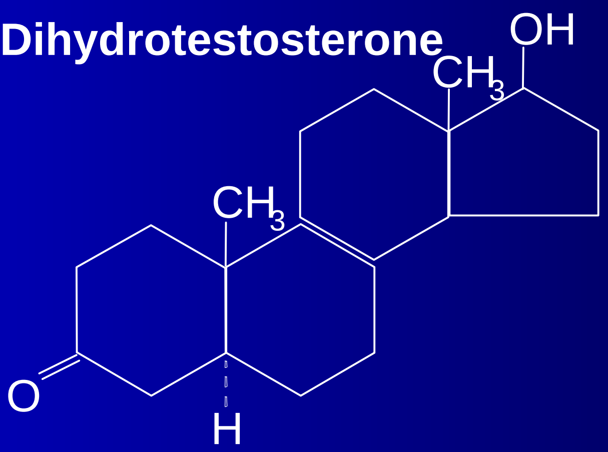
Progesterone



Testosterone



Dihydrotestosterone



Additional compounds to be evaluated in the future

- 4-nonylphenol (NP)

- 4-nonylphenol mono-ethoxylate (NP1EO)
- 4-nonylphenol diethoxylate (NP2EO)

- 4-nonylphenoxy carboxylic acid (NP1EC)
- 4-nonylphenoxy ethoxy carboxylic acid (NP2EC)

Technical approach

- Develop analytical methods to identify and quantify the target compounds. The approach will include concentration by solid-phase extraction, followed by LC/MS.

Analytical method for steroid compounds

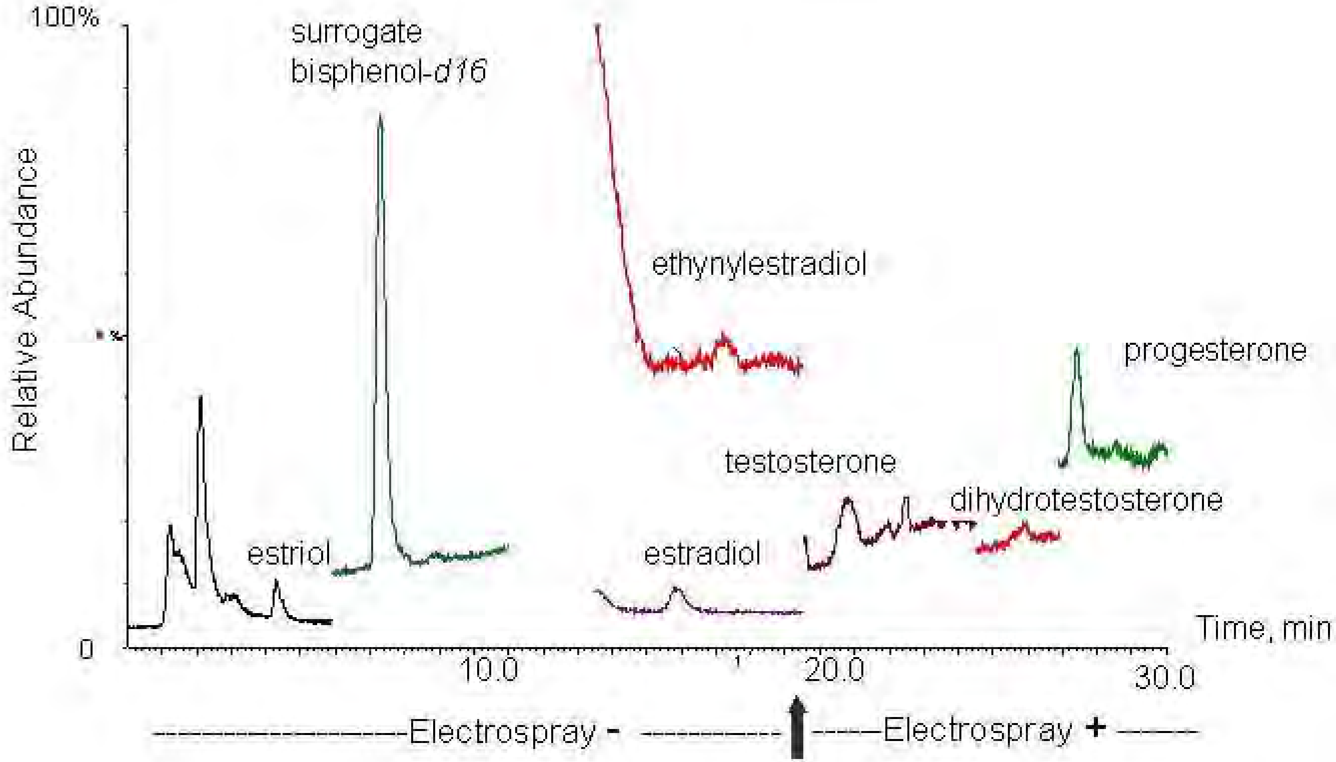
Solid phase extraction:

- Baker C18 XF speed disks eluted with methanol

Quantitation:

- Waters ZQ LC/MS, electrospray
- Xterra C18 column
- Single step gradient, 50 – 65% methanol in ammonium hydroxide in water
- Single ion mode

Single ion chromatograms of reagent water fortified at 1ng/L



Technical approach (cont.)

- Evaluate the use of a reporter gene assay, the MVLN assay, to detect the presence/ removal of estrogenic activity. This assay uses a human breast cell line (MCF-7) which has been stably transfected with the firefly luciferase gene.

Technical approach (cont.)

- Conduct bench-scale evaluations of various drinking water treatment technologies, including conventional treatment, granular activated carbon, softening and nanofiltration.

- Pilot-scale evaluations may be conducted on the treatment technologies that appear promising at bench-scale.

This study will provide information on:

- currently available drinking water treatment technologies that can remove EDCs, specifically the steroid hormones and the nonylphenolic compounds.

- approaches to optimize these treatment technologies for EDC removal.

- the need for additional management tools to be developed for the removal of EDCs during drinking water treatment.

Effective Risk Management of Endocrine Disrupting Chemicals, 29-30 January 2002, Cincinnati, Ohio USA

"European Community Strategy for Endocrine Disrupters: Implementation to date"

Kathryn Tierney,
European Commission, Environment DG

Contents of presentation

European Commission - DG ENV

1. **European Community (EC) Strategy for Endocrine Disrupters COM(1999)706**
2. **Implementation to date COM(2001)262**
   - *Identification of substances*
   - *R&D*
   - *Legislative Action*
3. **Conclusions**

1. EC Strategy (1)

European Commission - DG ENV

- Need for further research
- Need for international cooperation
- Need for communication to the public
- Need for appropriate policy action

1. EC Strategy (2)

European Commission - DG ENV

Short-term actions

- Establishment of priority list of substances for further evaluation of their role in endocrine disruption
- Use of existing legislative instruments
- Establishment of monitoring programmes
- Identification of specific cases of consumer use
- Information exchange/international cooperation
- Communication to the public
- Consultation with stakeholders

1. EC Strategy (3)

European Commission - DG ENV

Medium-term action

- Identification and assessment of EDs
- R&D
- Identification of substitutes

Long-term action

- Overall chemicals policy
- Water Framework Directive
- Other legislative actions/proposals

Identification of substances

- **Step 1 : Study Report by BKH, June 2000**
- **Step 2 : Consultation of scientists, Member States and other stakeholders**

$\Downarrow$

Priority list of actions

Identification of substances

- **BKH Report a reasonable starting point**
- **Focus on man-made chemicals plus synthetic /natural hormones present in the environment**
- **All 553 candidate substances retained for further evaluation plus 9 natural/synthetic hormones**
- **Need to set up iterative mechanism for updating**
- **No duplication of work with ongoing risk assessments under existing Community legislation**

Identification of substances

- **Of 118 substances with evidence of endocrine disruption or potential ED in BKH Report, 109 are already subject to regulatory measures:**
  - **16 obsolete plant protection products**
  - **14 banned in EU**
  - **22 with marketing restrictions in EU**
  - **11 subject to emission controls**
  - **46 currently on priority lists for risk assessment**

2. Implementation to date (4)

European Commission - DG ENV

Identification of substances

- *Study to conduct in-depth evaluation of 12 substances not currently addressed (12-18 months)*
- *Study to gather information on 435 substances with insufficient data in BKH Report (12-18 months)*
- *Invite Member States (MS) to take ED into account during RA of 46 substances (1-4 years)*
- *Invite MS to carry out classification on 2 substances (1-2 years)*

2. Implementation to date (5)

European Commission - DG ENV

R&D

- **4th Community Framework Programme for R&D (1994-1998) -- circa 8 M €/7 M $**
- **5th Community FP for R&D (1999-2002)**
  -- circa 10.45 M €/9.2 M $
  -- circa 20 M €/17.6 M $
  to be awarded in 2002

2. Implementation to date (6)

European Commission - DG ENV

Legislative action

Overall chemicals policy

- **White Paper, 13 February 2001**
  - **Authorisation procedure for CMR/POPs**
  - **Highlights need for further research on EDs -- on test methods, low dose effects, QSARs**
  - **Rigorous testing for long-term effects for >100t**

2. Implementation to date (7)

European Commission - DG ENV

Legislative action

Overall chemicals policy cont'd

- **Council Conclusions, 7 June 2001**
  - **Known EDs should be subject to authorisation when agreed scientifically valid test methods and criteria are established**

2. Implementation to date (8)

European Commission - DG ENV

Legislative action

Water Framework Directive

- **Directive 2000/60/EC and Decision 2455/2001/EC establishing the list of priority substances in the field of Water Policy**
  - **33 priority substances, 11 are candidate EDs**
  - **Measures to be proposed within 2 years aimed at ending or phasing out emissions, discharges and losses within 20 years.**

2. Implementation to date (9)

European Commission - DG ENV

Legislative action

General Product Safety Directive

- **Key risk management instrument in short-term**
- **Recent revision allows simplification of procedures and conditions for urgent measures at Community level**

3. Conclusions

European Commission - DG ENV

1999 Preparation of Community Strategy

2000-2001 Preparatory activities

identification of substances,

planning phases for R&D call for proposals

carrying out of technical studies

2001-2002 Implementation

launch of research projects

revision and preparation of legislative proposals

launch of new activities e.g. monitoring

Website address:

http://www.europa.eu.int/comm/environment/docum/99706sm.htm

Endocrine/Estrogen Letter

Online Enterprises • 245 Visitacion Ave, Brisbane, CA 94005
Phone: (415) 467-8779 • Fax 415-468-3144• e-mail: info@eeletter.com

Vol. 8, No. 2 (163)

February 2002

Endocrine/Estrogen Letter is published 12 times a year.

George Lawton,
Publisher and Executive Editor.
Joan McGrane, Associate Editor
Steve Vogel, Associate Editor
Toby Zambetti,
Director of Subscriber Management

ISBN # 1534-8830
Subscription price: $515 per year delivered by email

For editorial call: 415-467-8779
or email: glawton@eeletter.com

For subscription information and customer service call: 415-468-8112, or e-mail: toby@eeletter.com

E/E Conference Report

This special issue is dedicated to coverage of The Effective Risk Management of Endocrine Disrupting Chemical (EDC) workshop held in Cincinnati from January 29-30. The conference explored a number of issues including the context of risk management of EDCs, effects of EDCs on humans and wildlife, exposure assessment of EDCs, drinking water treatment, concentrated animal feed operations, waste water treatment, and the search for EDCs in combustion sources.

Greg Sayles, with the US EPA National Risk Management Research Laboratory (NRMRL) said this was the first technical meeting to focus on the risk management of endocrine disrupting chemicals.

There was some speculation at the conference that moving to a discussion on risk management is a bit premature, considering that the work on confirming the endocrine disrupting properties of chemicals is still in its infancy. But developing risk management strategies now may make it easier to deal with any confirmed endocrine disruptors once they have been conclusively identified.

Lee Mulkey with the NRMRL said there were three main things that were different about the topic and how they went about organizing the work.

First, EDCs have been identified as an emerging risk. Mulkey explained, "This is an important idea because the EPA typically responds and reacts to issues that come up, whether it is rivers that burn or the superfund being spun out of Love Canal. This is an issue where the EPA has had the foresight to consider what may or may not be an initiative that requires new policy development.

Second, we need to worry about the footprints of EDCs. There has not been much thought on EDCs as wastes, or how the environment can assimilate them.

Third, the idea of emerging risk is an integrative science. The idea is to develop knowledge about risk management while we are still learning about the effects and extent of exposure of EDCs. We can figure out ways in which intervention might be appropriate and the costs and benefits of various approaches.

Dr. Hugh McKinnon, Associate Director for Health, at the NRMRL talked about the history of risk management within the EPA. In 1983, the National Research Council put together the "Red Book" which spells out the risk assessment/risk management paradigm. It talks about the process of risk assessment, risk management, and risk

(Continued on page 2)

E/E Conference Report

(Continued from page 1)

communication.

Risk assessment classically has consisted of four steps: exposure assessment, hazard identification, dose response assessment, and risk characterization. The primary problem in risk assessment has been dealing with uncertainty.

McKinnon said where legal or political mandates are clear, or where we have unambiguous present risk, we will tolerate a great deal of uncertainty in risk assessment and remediation, especially if the costs are not high. It often turns out that as we develop a risk assessment, we get more data. We are able to reduce that uncertainty to a point where we can make a decision and hopefully that results in a lowering of risk. In an ideal case, if we have similar information about risk management options and their impact, then we can optimize our decision-making.

Mulkey discussed the development of a risk management evaluation, a summary of what is known about risk management options when a decision needs to be made. It includes an analysis of sources of risk, the risk management options that exist, and the availability, cost, and effectiveness of those options.

McKinnon said, "It was not our intent to second guess and rehash the risk assessment, it was to look at the risk management side. But in order to do that, we need to provide at least a capsule summary of what we know about risk. The goal is to reduce uncertainty in risk management for the EPA and others in order to provide cost effective risk management options."

Elements in risk management evaluation include identification, source strength, timing, and emission factors. Risk management options include pollution prevention, source control techniques, management practices, remediation, resorption, and adaptation.

Resources:
Greg Sayles: 513-569-7607 / sayles.gregory@epa.gov
Lee Mulkey: 513-569-7689 / Mulkey.lee@epa.gov
Hugh McKinnon: 513-569-7689 / mckinnon.hugh@epa.gov

European Community Risk Management Strategy

Kathryn Tierney, Principal Administrator with the European Commission (EC), called in from Brussels with a report on, "European Community Strategy for Endocrine Disrupters: Implementation to Date." She said the basic strategy towards EDCs was adopted by the EC in 1999 and consisted of 3 elements: identifying substances, research & development, and legislative action.

Tierney stressed that the strategy was not just imposed on industry. She explained, "We have to have buy in from industry, health and consumer protection, and agriculture, and when the commission comes up with a strategy like this we have extensive consultations with member states, NGOs and others.

The core of the EC strategy is the development of test methods and research. She noted, "When we adopted this strategy in 1999, the research was still going in. We are still waiting for agreed test methods. We wanted to look at what we can do when we have agreed test methods."

Short-term actions the EC is conducting while waiting for agreed test methods to be developed and validated include:

1. Establishment of a prior list of substances for evaluation of their role in endocrine disruption.
2. The use of existing legislative instruments. Current legislation now provides 32 main instruments that can be used to deal with problem chemicals. One is classification where substances have to be subject to existing test methods. Even if they have not been demonstrated to be EDCs, substances can also be regulated based on their effects as carcinogens or for causing reproductive disorders. Tierney said, "Even though we don't have an agreed idea of what constitutes an EDC, we should still take whatever evidence there is into account. We don't want to bury our head in the sand and ignore the evidence

(Continued on next page)

E/E Conference Report

EC Risk Management Strategy

(Continued from page 2)

simply because we don't have an agreed upon test method."

3. Establishment of monitoring programs. This is not necessarily doing new monitoring, but looking at what is already out there.
4. Identification of specific cases of consumer use. Tierney noted, "Here we sensed that if we found in the preparatory work specific substances of concern to consumers we should not wait for agreed upon test methods to see what we can do to reduce the risk. I am talking about cases where you have vulnerable people like children."
5. Information exchange and cooperation with international colleagues in order not to duplicate the same work.
6. Communication with the public. Tierney said, "This was important in 1999 when the strategy was published and a lot of media reports were turning up fear."
7. Consulting with stakeholders in order to insert feedback into the process.

Medium-term action of the EC includes:

1. Identification and assessment of EDCs and the development of test methods.
2. Research and development
3. Identification of substitutes. Tierney said they put that as a medium term objective rather than a short term one because they weren't sure if the substitutes could result in the same fears as the chemicals being replaced.

Long-term action includes:

1. Overhaul of the overall chemicals policy.
2. The water framework directive adopted in 2000, which overhauled much of the EU legislation on water.
3. Other legislative actions and proposals.

The strategy adopted by the EC was discussed with the European Council and the European Parliament. Tierney said the Council, consisting of the EU member states, stressed the need to apply the precautionary principle and the need for quick and effective risk management policies. The parliament was even stronger to some extent in criticizing the commission strategy as not being aggressive enough.

Tierney also talked about EC work on identifying suspected EDCs. The EC hired BKH consulting in the Netherlands to put together a report of chemicals named by Greenpeace, Germany, US, and the scientific literature, They came up with an initial list of 553 substances.

Tierney said, "When the study was launched, it was not expected we would come up with over 500 chemicals. Within that study we had to whittle down and focus on high production highly persistent substances. So we gathered more data on those and finally looked at exposure."

"Even though we don't have an agreed idea of what constitutes an EDC, we should still take whatever evidence there is into account. We don't want to bury our head in the sand and ignore the evidence simply because we don't have an agreed upon test method." -- Kathryn Tierney

Quite a bit of misinformation was spawned by this effort. For example, it was widely reported that the EU had established a priority list of EDCs. Tierney said, "This is not the case. The second part of the process was to consult widely on the list. We have a commitment to ecotoxicity. We consult with the World Health Organization and the OECD to decide what we should focus our resources on."

The actual results of the BKH report were seen as a reasonable starting point. Tierney noted, "We were looking at a starting point where he have to further evaluate the substances. We are only at the beginning."

The focus of the list was on manmade chemicals and synthetic and natural hormones present in the environment. All 553 substances were retained for further evaluation plus 9 natural/synthetic hormones. None have dropped off the list. Tierney said, "We need a mechanism to update the report so as we get to know more and new evidence comes in,

(Continued on next page)

E/E Conference Report

EC Risk Management Strategy

(Continued from page 3)

some will drop in concern and others will rise in concern."

An important part of the EC's strategy is to not duplicate existing community legislation. When they looked at high production and highly persistent chemicals, they found that 118 had evidence or potential evidence of ED.

109 of these were already subject to existing regulatory measures.

16 were obsolete plant protection products.

14 were already banned from the EU.

22 were subject to marketing restrictions in the EU.

11 were subject to emission controls.

46 were on priority lists for risk assessment. 31 of these are being risk assessed currently and 15 are plant protection products or pesticides that are also being risk assessed.

Tierney said, "We feel that it is an important result that these substances… are already on black lists for other reasons. For communications this is important. Even if we don't know everything about the ED ability, many are already blacklisted."

None of these chemicals drop off the list. Tierney explained, "When we do have agreed test methods, we may decide the some of the substances need a closer look. That is why none of the candidates dropped off the table."

Following the consultation process and the result of this report, the EU decided to get away from the idea of coming up with a priority list of substances. The main reason is that the EU legislation has a strict connotation of priority and risk assessment. There is no EU legislation to address EDCs specifically. So they moved away from a priority list of chemicals and came up with a priority list of action.

Out of the other 435 chemicals, they found 12 substances that were not addressed by legislation for any reason. The EC launched a study of these in November 2001 that goes through May 2002 to look for any evidence that they are EDCs. The EC has also launched a second study of all of the 435 substances with insufficient data in the BKH report in November 2001 that will run through August 2002.

The EC decided to invite member states to take EDCs into account during risk assessment and invited them to carry out classification of 2 substances they found that were not classified. Tierney said this means they can carry out or make a request that these two substances be tested with existing test methods.

Tierney also discussed the R&D initiatives being sponsored by the government. The 4th Community Framework Programme (CFP) for R&D (1994-1998) spent 8 million euro ($7 million) on endocrine disruptors, which complemented other money spent by industry. The 5th CFP for R&D (1999-2002) budgeted 10.5 million euros ($9.2 million). There is also another 10 million euros being spent on substances suspected of being EDCs like PCBs, but the focus is not on endocrine disruption. The 5th CFP includes another 20 million euros to be awarded in 2002 towards suspected EDC research, which does not include industry contributions. These figures only include European Community funding and don't include money going into national programs in Denmark, Germany, and the UK.

Legislative strategy is being looked at by the EC as a long-term action. The overall chemical policy in Europe is being overhauled. The old system allowed existing substances to be authorized for use unless specifically forbidden. Last February, a white paper came out advocating that substances that are carcinogenic, mutagenic or reprotoxic (CMR) would be forbidden unless specifically authorized. The existing substances include over 30,000 chemicals with annual production greater than 1 ton. Rigorous tests for long-term health effects were recommended for chemicals produced

(Continued on next page)

E/E Conference Report

EC Risk Management Strategy

(Continued from page 4)

in quantities greater than 100 tons. Commentary in the white paper on endocrine disruptors themselves highlighted the need for further research on endocrine disruptors, test methods, low dose effects and Quantitative Structural-Analysis Relationships (QSAR).

The white paper was adopted by the EC and the council adopted the conclusions in June. One of the things they highlighted is that they know that EDCs should be subject to authorization. Now the EC has established various working groups where they are looking at endocrine disruption. The commission now has to prepare draft legislation where they have to give advice on how the EU will incorporate EDCs into the chemicals policy.

Tierney said, "Up until now, we have had a parallel EDC process, but the goal is that anything that we do with EDCs should be consistent with the overall chemical policy."

The original water framework directive was adopted in 2000. Tierney said that the EC has adopted a list of 33 priority substances, and of these 11 are candidate EDCs. When they identified these priority substances, any evidence of endocrine disruption was considered, but there were many other very good reasons to include them. Measurements have to be reported back within 2 years with the aim of ending or phasing out emissions, discharges and losses within 20 years.

Another piece of relevant legislation is the General Product Safety Directive. Tierney said, "This is the only instrument we have that can be used from an emergency point of view in the short term." It has been used to create short-term restrictions on the use of phthalates in toys.

Tierney noted, "We have placed a special focus on EDCs with this strategy and that has allowed us to increase the attention given to it in existing legislation. The EU parliament has recommended banning EDCs. But the commission is making sure the whole process is taken up in the existing process and will be part of the overall chemical policy rather than proposing new legislation to deal with EDCs."

Tierney concluded, "The main thing we need to push is for agreed test methods. Our strategy is that until we have agreed test methods, we cannot take legislative action to reduce the risk. In the mean time, if we come up with some specific cases, then we can take specific action and use emergency measures for a temporary withdrawal. But that is a specific case for consumer use. Without agreed upon test methods, we can only act on a case-by-case basis.

William Owens, with Proctor & Gamble, noted that we have known that many endocrine mechanisms produce reproductive and developmental effects and that Europe is talking about an authorization system for reproductive and development toxins. He then asked, "How are you going to work a subset of endocrine disruptors into the system so you don't have duplication and conflicts and inconsistencies?"

Tierney responded, "It is true we have CMRs. We don't do risk assessments. We just place restrictions on consumer use. There is a fair amount of controversy over whether EDCs need a hazard category of their own. We know that most of the effects are on the reproductive system. There are 2 things. One is whether the substances have been through this. That means that the instructions we now have will have to be relooked at. Also there is a possibility there are other effects."

Resources:
http://www.europa.eu.int/comm/environment/docum/99706sm.htm
Kathryn Tierney: 011-322-296-8118 / Kathryn.Tierney@cec.eu.int

Correction: Last months E/E Letter should have reported the number of animals used for endocrine disruptor testing could potentially climb to 96 million animals if all approximately 80,000 chemicals under consideration for screening are tested.

E/E Conference Report

Effects of EDCs On Wildlife

Dr. Gary Ankley, Chief of the US EPA's Min-Continent Ecology Division, Toxic Effects Characterization Branch, in Duluth, MN discussed an "Overview of Effects and Assessment of Endocrine-Disrupting Chemicals in Wildlife." He said there are a number of implications of EDC effects in wildlife. There are a number of populations that are experiencing serious decline for which we don't have a cause. It is also theorized that EDCs can result in community level effects by affecting key species. The indirect implications are that EDCs can result in economic issues like reductions in fishing and that these animals could be acting as sentinels for human health effects.

But Ankley cautioned that we should not jump to conclusions that all wildlife deformities are caused by EDCs. For example, a lot of people are concerned about the implications of deformed frogs on their children's health. But he said there is strong evidence that some of these effects are caused by other factors such as parasites or UV radiation rather than EDCs.

With a few notable exceptions, such as DES, documentation of EDC effects in humans is rare. Ankley said this is not true in wildlife. There is evidence of EDC effects on invertebrates, fish, frogs, reptiles, birds, and mammals.

EDCs have been suspected in causing:

-Hermaphrodism in gastropods

-Feminization or masculinization in fish

-Malformations in amphibians

-Feminization of alligators

-Developmental lethalities/abnormalities in great lakes fish and birds

-Feminization of mammalian carnivores like mink and otters

There is evidence that tributyltin is causing imposex (the simultaneous presence of male and female genitalia) in gastropods such as snails. This resulted in sterility and marked declines for specific populations. It appears the metabolic pathway being affected is the aromatase enzyme used for converting testosterone into estradiol. Ankley noted, "What is really neat about this is that TBT levels are going down and we are starting to see the population going up, so we have a complete case history with regards to these chemicals.

With regards to fish, the evidence started coming in the early 1990s from John Sumpter's group in the UK that fish gonads had both male and female characteristics. Ankley said that since the initial discovery in the UK, there have been a lot of studies on municipal discharges in the UK, US, Germany, and other countries.

Ankley noted that at least in the UK, the changes seem to be due to natural and synthetic estrogens such as estradiol. He said, "Here we have an interesting situation where our pharmaceutical products could be causing this. There has also been a focus on alkylphenols. I don't think that is the case in municipal effluents, but in the case of industrial effluents, it could be more common."

Another problem that has been noted is the masculinization of fish near the effluent of pulp and paper mills in the US, Canada and Scandinavia. Ankley said it looks like these are caused by phytoandrogens that are normally bound into the wood, and then released during paper manufacture. This is because the same androgenic effect has been seen at several mills independently of the underlying chemistry used to make the paper.

Ankley discussed assays they have been working on in Duluth that could be used for people involved in risk management decisions of EDCs. One is the short-term reproductive assay of the fathead minnow, the "White Rat" of aquatic toxicology. The strengths of this assay are

-There are standard methods for culturing and holding them at different life stages,

-There is a history of use in the regulatory community

-They are easily handled and cultured

-They are small but still large enough to collect various tissues from

-They have a relatively rapid life cycle

-They are serial spawners (females spawn

(Continued on next page)

E/E Conference Report

Effects of EDCs On Wildlife

(Continued from paged 6)

every 3 days) so you can get an estimate of fecundity.

-They are a dimorphic species (the male is bigger and has a darker color)

Ankley said they want a short term, cost effective assay that could be successfully implemented by a range of labs. They also wanted an assay that would detect and discriminate among EDC modes of action of concern: estrogen/anti-estrogen, androgen/anti-androgen, and modulators of sex steroid metabolism. They also wanted to incorporate measurements of fecundity, fertility, hatch, and early survival.

Key endpoints from an EDC perspective include secondary sex characteristics such as gonadal weight, plasma vitellogenin, and plasma steroids.

Most exposure routes occur by adding a chemical to the water, diet, or injection. Ankley said injection is a little tricky to do at first, but it is very effective when you are working with small quantities of expensive test chemicals, of if you don't want to release them into the environment.

In developing a particular assay, it is important to run it through a variety of known chemicals to establish a baseline level of effects. Ankley recommended:

Beta estradiol – Estrogen Receptor (ER) agonist
Methoxychlor – ER agonist
ZM 189,154 – ER antagonist
Trenbolone – Androgen Receptor (AR) agonist
Methyltestosterone – AR agonist
Vinclozolin – AR antagonist
Flutamide – AR antagonist
Fadrozole – inhibitor of steroid metabolism

Trenbolone is a compound used in animal feedlot operations to build muscle mass. A recent Environmental Health Information Service report noted that much of it passes through the animal and has a half-life on the order of 260 days, so it could be involved in runoff. Vinclozolin is an organochlorine fungicide. The parent chemical does not create an effect but 2 of its metabolites do. Fadrozole is a chemical used to treat breast cancer. It appears to bind to aromatase, the enzyme that converts testosterone to estradiol.

Ankley concluded, "We feel we have a system that effectively identifies and discriminates among the EDC modes of concern. Another important aspect is that it enables routine collection of data (fecundity, fertility, and hatch) of utility to higher tier risk assessments. The assays being developed applied to both the single chemical and complex mixture testing."

David Lattier, with the US EPA discussed the "Development of Biological Methods to Characterize Exposure of Wildlife To EDCs." He said they have developed a test for measuring estrogen in the field based on gene expression. Most of the work was done with single gene indicators using single chemicals to look at these genes and when they are activated. The initial work focused on fathead minnows and the expression of the egg protein vitellogenin.

The research used estradiol, ethinylestradiol, and DEHP to cause the gene expression. When the estrogen enters the cell membrane, it binds with the estrogen receptor, resulting in gene activation.

Lattier said the test has identified vitellogenin at levels of chemical as low as 2 ng/l within 8 hours of exposure. By looking for the gene rather than the expressed vitellogenin, they are able to attain a higher level of consistency. Lattier said the standards of deviation are considerably higher for the detection of the protein than for the gene that makes the protein.

Lattier said that his group wanted to put the single gene test into the hands of the EPA regions. He noted, "We will do a demonstration in your backyard, but you must provide the food."

Myriam Medina-Vera discussed the "Development of Chemical Methods to Characterize Exposure to EDCs in the Neuse River Basin" in North Carolina. She said the Neuse River basin is a highly industrial area with about 1.5 million human inhabitants and a substantial hog population.

Some of the suspected EDCs being studied in

E/E Conference Report

Effects of EDCs On Wildlife

(Continued from page 7)

the region include alkylphenol polyethoxylates, antibiotics, pesticides, and metals including arsenic, cadmium, copper, manganese, lead, and tin.

She said the alkylphenol polyethoxylate research is a hot area and that a lot of countries are trying to decide whether to regulate them. Some of the variants are persistent, accumulate in fish, and can link to the estrogen receptor. They are used in making pulp and paper, plastics, leather, agriculture, household, industrial and institutional cleaning products, and as a spermicide in contraceptive jellies and creams. The US is one of the largest producers and consumers.

The degradation mechanism of these chemicals is of interest, because Medina-Vera noted, wastewater treatment plants are only capable of degrading 30-35% of the nonylphenol. She said the controversy is that the combination of the monoethoxylates and acetic acid congeners may be more toxic than nonylphenol. She pondered, "How do you define the toxicity of chemicals you are looking at. The first question they are going to ask you is which ones are the toxic ones, which are the ones you really want to control."

Resources:

Gary Ankley:
218-529-5147 / ankley.gerald@epa.gov
Myriam Medina-Vera:
919-541-5016 / medina-vera.myriam@epa.gov

EPA's EDC Strategy

Elaine Z. Francis, National Program Director for Endocrine Disruptors Research at the US EPA, spoke about "EPA's Endocrine Disruptors Screening Program: Legislation, Implementation, and Research." She outlined the basis for the EPA's EDC mandate from the 1996 Food Quality Protection Act and Safe Water Drinking Act. These specified that the EPA must develop a program in August 1998, implement the program in 1998, and report to congress in 2000, although the schedule has slipped considerably. The mandates did provide some discretionary authority. For example, the EPA can require screening and testing of:

-Any active or inert pesticide ingredient

-Any chemical that has an effect cumulative to an effect of a pesticide

-Drinking water source contaminant

- Other endocrine effects

- Environmental effects

A snapshot of the chemicals that need to be looked at include:

-900 pesticide active ingredients

-Inert ingredient found in various combinations with pesticide active ingredients

-Approximately 75,500 industrial chemicals in the Toxic Substances Control Act. This does not include metals or sanitation byproducts.

The proposed phase 1 of the testing program is to focus on pesticide active ingredient and high production volume chemicals. Francis said, "It is a fairly reasonable size of chemicals (1500-1600) to begin screening."

There are a number of factors that go into deciding the priority of a particular chemical such as persistence, presence in sediments, presence in food and water, and bioconcentration. Francis noted, "For the most part, we lack a lot of information in terms of the effects for this priority setting. There are two solutions we have come up with."

One approach was to use High Throughput Screen (HTPS) technology. The EPA thought it would run thousands of chemicals through to see where they fall for the remainder of the Tier 1 screen. Francis said, "We did do HTPS, but felt that it was unreliable for regulatory use. We are now focusing on the second solution, QSAR." However, both approaches need further validation, which is mandated by law.

Francis said Tier 1 validation is under way, and should be done by 2004, when Phase 1 of testing will begin. Tier II validation will continue through 2005.

E/E Conference Report

Exposure Assessment of Suspected EDCs

One of the missing elements in the debate over EDCs is data about the levels of chemicals humans and wildlife are exposed to. Although many chemicals demonstrate some ability to affect hormones, there is not much cause for concern if exposure is low. A number of researchers presented information about research in this area.

Steven Goodbred, with USGS talked about "Monitoring Endocrine Disrupting Compounds In Aquatic Ecosystems in the US." He said there is no systematic knowledge of EDCs in the US due to 1) an incomplete consensus on EDCs, 2) analytical methods are still being developed for many compounds, 3) difficulty selecting a sampling matrix, and 4) major funding is needed.

NAWQA Study Results

Matrix	Suspected EDCs
Water for hydrophilic pesticides	20
Bed sediment for PAH and phenols	17
Fish Tissue for organochlorines and PCBs	24

Several agencies have national monitoring programs that include EDCs. The USGS has NAWQA, TOXICS, and BEST. The EPA has EMAP, REMAP, and the National Dioxin Study. NOAA has Mussel Watch and Benthic Surveillance. The FDA has the National Monitoring Program for Food and Feed.

The USGS launched the National Water Quality Assessment Program (NAWQA) to assess the status and trends of surface and ground waters throughout the US. It surveys 60 basins throughout the US and cycles through them every 4 years. Goodbred said, "We are looking at factors that affect water quality including EDCs."

In the urban water samples analyzed, they found that 100% of the fish had pesticide exposure and 99% had some pesticides. In agricultural areas, 85% of the fish had been exposed to pesticides and 92% of the water samples contained them. 33% of the major aquifers tested positive for pesticides.

Pesticides were found to almost always occur as mixtures. About 50% of the sites tested had at least 6 different pesticides. Goodbred said, "Organisms are exposed to very complex mixtures, and this is something we need to deal with for risk assessment."

One issue is that pesticides in streams usually occur in strong seasonal pulses. Goodbred noted, "If you are not out there sampling daily during storm surges, you are going to miss a lot of information." Although the average concentrations are far below the maximum levels allowed, individual peaks are much higher.

Another issue is that the breakdown products can be 10-25 times the concentration of the parent compound. Goodbred said, "We really need to understand the kind of breakdown products and whether they are endocrine disruptors. That could keep chemists busy for the next millennium."

Goodbred concluded, "It appears a lot of EDCs are potentially widespread in the US. That is not a surprise, but it is interesting and something we need to deal with for risk assessments."

The USGS is preparing to release the National Reconnaissance of Pharmaceutical and Other Emerging Contaminants in US Streams this coming March. The study will try to determine if pharmaceuticals, antibiotics, hormones and other chemicals consumed by humans and animals are entering the environment. The approach is to develop sensitive and specific analytical methods for 22 antibiotics, 14 prescription drugs, 5 non-prescription drugs, 15 hormones and steroids, and 39 household and industrial compounds.

Phase I was a reconnaissance of 139 streams in 30 states broken down into agriculture, urban, mixed land use, and minimally developed groups. The preliminary data indicated that 82 out of 95 wastewater compounds looked for were found in 80% of the stream samples. Detection of multiple compounds was common. About 75% had more than one, and about 35% had more than 10. The steroids had one of the highest concentrations, but

(Continued on next page)

E/E Conference Report

Exposure Assessment

(Continued from page 9)

nonylphenol and its ethoxylates also ranked high.

Goodbred reiterated that the USGS has a lot of quality-controlled data, and is willing to make it available to anyone who wants it free of charge.

Ruthann Rudel, a senior environmental toxicologist at the Silent Spring Institute discussed "Residential Indoor Air and Dust Measurements of Phthalates and other Endocrine Disrupting Compounds." Rudel said, "There are a large number of toxicologically important chemicals that are widespread in indoor environments."

She has been involved in an epidemiological study of 2100 people in Cape Cod, MA. As part of that study, her team also been looking at the levels of 90 suspected EDCs found in blood, urine, dust, and air in a subset of 120 of the test subjects. She presented data on 30 of these subjects. The subjects were primarily older women over 65 years old. It included individuals who reported both low and high uses of pesticides.

Rudel said exposure assessment is a weak element in environmental epidemiology, and that the government accounting office has issued a couple of reports citing this weakness. Exposure data could help to identify highly exposed populations.

The preliminary data showed the most abundant chemical in the air was o-phenylphenol. Other common chemicals included Di-Butyl Phthalate, Di Ethyl Phthalate, pentachlorophenol, diazinon, heptachlor, methoxychlor, folpet, piperonyl, butoxide, carbaryl, permethrin, and chlordane. Rudel said, "I was interested in how many banned pesticides were still in air samples."

They also found that indoor air concentrations of suspected EDCs were sometimes 100 times greater than in outdoor concentrations. The team also decided to look at dust, which Rudel described as, "a good reservoir reflecting current and past use of chemicals in the house." Dust also presents an exposure pathway for children. They found phthalates are clearly the most abundant in dust. Pesticides found in the dust included chlordane, dieldrin, and DDT.

They also looked at EPA Preliminary Remediation Goals for available compounds and compared these to the results. In general the samples were below these levels with the exception of chlordane, heptachlor, and PCB52.

Rudel said a striking thing about the results was that the profile in urine and air samples looked similar to each other. "If you take these exposure assessments, you would not find that these air concentrations would provide any significant dose. One hypothesis is that the ambient concentration indoors could be proxies for various exposures, such as putting on hair spray."

Rudel concluded her presentation, "We are currently beginning the analysis for our full set of data. We are looking forward to interesting and informative findings and we are seeking support for additional work and collaboration in this area."

Resources:
Nawqa: http://water.usgs.gov/nawqa/
National Reconnaissance of Emerging Contaminants in the Nation's Water Resources:
http://toxics.usgs.gov/regional/emc.html
Steven Goodbred:
916-278-3097 / goodbred@usgs.gov
Ruthann Rudel:
617-332-4288 / rudel@silentspring.org

Rumor Mill

A source told E/E Letter that legal action might be forthcoming around a case in which Frederick Vom Saal alleges Dow Chemical tried to bribe him to suppress data around the low-dose effects of Bisphenol-A. However, Vom Saal responded, "I wrote a letter to the FDA about it, but I have no desire to spend any more time pursuing this case."

E/E Conference Report

Risk Management Approaches

Greg Sayles, a chemical engineer with the US EPA's National Risk Management Research Laboratory discussed "EPA's Risk Management Evaluation (RME) of EDCs." An RME summarizes the current understanding of risk management of a particular environmental challenge and includes data about known health and ecological effects, significant sources to the environment, and significant exposures and environmental sinks. The RME on suspected EDCs includes chapters on a number of chemical classes including alkylphenol ethoxylates, DDT and DDE, natural, veterinary, and pharmaceutical steroid hormones, PCBs, chlorinated dioxins, and furans.

The RME for EDCs identifies currently available risk management approaches that were developed for other purposes, but appear useful in managing the risk of EDCs. The document also indicates where new risk management approaches are needed. The RME will be useful 1) to inform risk managers such as regulators on what technical approaches are currently available for managing EDC risk, 2) to educate the public about what risk management approaches are and are not available now, 3) to motivate environmental consultants/ engineers to review current skills or to develop new skills applicable to managing exposure to EDCs, and 4) to guide risk management researchers, such as NRMRL, in planning EDC risk management research programs.

After the presentation, Mark Walton, Business Public Issue Leader, at Dow Plastics noted that these kinds of lists from a regulatory agency could be exciting to the media because they can be taken out of context. He asked how the EPA planned to communicate information about these suspected EDCs.

Sayles responded, "When you see the document you will see a fair amount of caveats around that list. They are very practical caveats. You cannot work on every known chemical forever and try and get a document done. We are very clear these are compounds still under study. We have made that very clear."

Vincent Kramer, with Dow AgroSciences commented, "Every time you consider a substitute for a product, you need to consider the risk of removal of that benefit. In the area of growth promoters for cattle, it could be argued that they help supply a meat product that is affordable to the general population that has benefits for nutrition. If you consider another way of growing cattle it may not include the fact that it may make the price of meat unaffordable."

Carolyn Acheson, a chemical engineer with NRMRL discussed "Using Bioassays to Evaluate The Performance of Risk Management Techniques." She started the presentation off noting, "One lesson I have learned is that you will have a much better understanding of your risk management technique if you include bioassays."

She said researchers in this area make simplifying assumptions that ignore important factors. A common assumption is that if we know the contaminant, we understand the risk. But a researcher may ignore the fact that there is an incomplete removal of, or the development of side products or that there are co-contaminants.

Another common assumption is that treatment reduces toxicity. But a researcher may ignore process amendments, other reactions or matrix changes. For example, they have learned when remediating gasoline that if you aerate the soil you can reduce contaminant concentrations, but you can also release metals.

If you are just using chemical assays to look for changes, you might miss these important side effects. By integrating a bioassay into the remediation process, you can notice these side effects, and then use chemical assays to pinpoint the chemicals and processes responsible.

Acheson described a case study where they were looking at remediating PCB from soil. After treatment, the levels of PCB were reduced, but a

(Continued on next page)

E/E Conference Report

Risk Management Approaches

(Continued from page 11)

bioassay revealed the soil was still quite toxic. It turned out that in the course of reducing PCB concentration, they had created propanol. So they added a rinse step, which reduced the total toxicity of the soil.

Four types of bioassays that the NRMRL has been exploring include: 1) sediment/aquatic invertebrate tests, 2) terrestrial invertebrate tests, 3) in-vitro tests and 4) seed germination and root elongation. The sediment aquatic research test uses a commonly studied aquatic organism but requires substantial lab equipment and takes about 1 month. The terrestrial invertebrate test uses an earthworm model. It has disadvantage that it cannot measure imposex effects because the test is not sensitive to androgens. The in-vitro assays include an e-screen based on mouse breast cells lines and yeast cells.

Resources:
Greg Sayles: 513-569-7607 / sayles.gregory@epa.gov
Carolyn Acheson 513-569-7190 / acheson.carolyn@epa.gov

Drinking Water Treatment

An important area of risk management for confirmed and suspected EDCs lies in removing them from the drinking water. If research indicates that existing treatment processes can ameliorate these chemicals sufficiently, then perhaps no other steps will be necessary. But if they don't, new processes will have to be developed for dealing with these chemicals, particularly if they are detected at high levels.

John Cicmanec, working with the National Risk Management Research Laboratory reported that a number of treatment processes had been tested for their efficacy in removing chemicals such as alkylphenols, bisphenol-A, phthalates, PCBs, dioxins, dibenzofurans, and the pesticides methoxychlor, endosulfan and atrazine. Cicmanec said a number of other vectors are on the horizon of concern including coxsackievirus (juvenile diabetes), heliobacter pylori (thyroid disorders), trenbolone acetate, and melengestrol acetate.

In addition to the conventional water treatment process of sedimentation, coagulation, and filtration, they also considered the effects of granular activated carbon (GAC), powdered activated carbon (PAC), nanofiltration, reverse osmosis, and air stripping for the removal of confirmed or suspected EDCs. Their findings indicate that GAC and PAC were the most effective at removing the chemicals tested.

Cicmanec spoke about nonylphenol noting that after Sweden established regulations, nonylphenol rates decreased from 75 micrograms/l down to 9 micrograms/l within one year. He added that nonylphenol is found in 24% of the water samples from the US.

Looking at traditional water treatment techniques, Cicmanec said they found that by simply performing coagulation, they were able to remove 87% of methoxychlor and 98% of DDT. Other chemicals were harder to remove. For example, the traditional techniques only removed from 10-60% of lindane, aldrin, dieldrin & parathion. They also found that UV, which is used to kill microbes, might also have an effect in helping to break down chemicals.

PAC and GAC are the most effective techniques observed to date, but their costs keeps them beyond the reach of many plants. PAC is used in about 48% of the surface water plants, and 12% of the ground water plants. GAC is used in 12% of the surface water and 5% of ground water treatment plants.

Factors that affect the effectiveness of PAC/GAC include contaminant adsorbability, concentration, multi-component adsorption, adsorption kinetics, temperature (works best at low temp) carbon dose, and contact time.

In Cincinnati they added a PAC plant a few years ago at a cost of about $60 million, which amortizes out to a present cost of 27 cents/1,000 gallons versus 25 cents/1,000 gallons before.

Kathleen Schenck, an environmental scientist

(Continued on next page)

E/E Conference Report

Drinking Water

(Continued from page 12)

with the NRMRL discussed "Evaluating Drinking Water Treatment Technologies for Removal of EDCs. She said two basic strategies exist for decreasing the risks of potential adverse health effects from EDCs in drinking water. One is to protect source waters from contamination by EDCs. The other is to remove EDCs during the treatment process.

Schenk's team evaluated the removal of a number of compounds including estradiol, estriol, ethinylestradiol, progesterone, testosterone, and dihydrotestosterone. The project was divided into four parts. The first is the development of an analytical method to identify and quantify the analysis. The second is the application of a reporter gene MVLN assay to evaluate the removal of estrogenic activity from the water. It is designed to detect compounds that may have been created in treating the water. The third part is the use of bench scale evaluations of various drinking water processes. The final part will be to conduct pilot scale evaluations of these techniques, if warranted.

The project was launched in November 2000 and is still in process. Schenck said she had expected to see some engineering data by now, but the analysis is taking longer than expected. The steroid hormone analysis should be done within a year. Work has not even started on the nonylphenol compounds.

Schenck noted that at the moment, there are no initiatives to regulate any of the chemicals being tested. She said, "We don't even know if there is a human health risk associated with the presence of them. The reason we are doing this is that NRMRL has decided not to wait until every last decision is made on a risk assessment, but to go ahead and look at emerging problems and how to fix them." It may turn out that GAC is all that is required.

Fred Pontius, President of Pontius Water Consultants, in Lakewood CO discussed "Risk Management of EDCs in Drinking Water." He said there are four simple questions at the heart of the matter:

1. Are current regulations adequate?
2. Should we be concerned about unregulated EDCs such as pharmaceuticals?
3. What can water utilities do to manage risks?
4. What are the critical research questions?

Drinking water contamination is currently regulated by the Safe Drinking Water Act. Pontius said that we may not be able to regulate based on an endocrine disruptor effect, but in many cases, chemicals are covered by other types of effects. The EPA also has the latitude to monitor unregulated compounds of concern.

When a chemical is regulated, the EPA sets a maximum containment level (MCL) goal. But in 1996 the EPA was directed to determine if the benefits of an MCL justified the cost. Pontius said, "This is not going to be a straightforward proposition. We are learning that with arsenic."

Pontius believes that overall, current regulations are sufficient, even though the EPA has not started down the path of regulating EDCs.

With regards to unregulated contaminants, Pontius noted, "Sometimes we find them, but most of the time we don't. We need to recognize there are many types of chemicals and considerations. It is important to be careful with generalizations. If a regulation has been set that requires me to install a treatment system, I will not target the one chemical. I will try and get as much out of that as I can."

The critical research questions in this area include:
1) What are the relative health effects of EDCs to determine which chemicals are important to remove?
2) What is the occurrence, fate and transport of EDCs?
3) What is the treatment effectiveness on EDCs?

Resources:
John Cicmanec: 513-569-7481 / cicmanec.john@epa.gov
Kathleen Schenck: 513-569-7947 / schenck.kathleen@epa.gov
Frederick Pontius: 303-986-9923 / fredp@pontiuswater.com

E/E Conference Report

Concentrated Animal Feed Operations

A major source of environmental hormones are confined animal feed operations (CAFO) in which thousands of animals are housed, sometimes with substandard sewage treatment systems. The quantity of hormones and hormone mimics is increased by the use of hormones and antibiotics to increase growth, and substantial populations of pregnant or lactating animals. Researchers presented information on a number of different aspects of the concerns about how to manage the risks of these CAFOs.

Steven Hutchins, a research environmental scientist with the US EPA National Risk Management Research Laboratory presented a talk on the "Potential of CAFOs to Contribute Estrogens to the Environment."

There are many unknowns regarding the potential for CAFOs to contribute EDCs to the environment, due to the variety of CAFO operations, the diverse natures of potential EDCs themselves, and the different types of endocrine receptors that could be affected. Two projects have been initiated. One, to evaluate EDC activity from different types of CAFOs and the other to measure levels of estrogens in swine waste effluents.

The first project is being conducted by Oklahoma State University under a cooperative agreement and is designed to evaluate swine, dairy, and beef CAFO lagoons. Three analyses will be used to assess for the presence of EDCs: 1) Xenopus Tail Resorption Assay to measure for thyroid disruptors, 2) Enzyme Linked Immunoassay (ELISA) to detect estrogenic compounds in frogs, 3) A measurement of plasma testosterone and 17â-estradiol concentrations as an indicator of alteration in reproductive endocrine homeostasis.

The work is ongoing. Preliminary results indicated that although the swine effluent is quite toxic, none of the lagoons exhibited estrogenic activity.

In the second study, Man Tech Environmental Services is working under contract to optimize both ELISA and Liquid Chromatography/Mass Spectrometry/MS analytical procedures for the analysis of complex wastewaters. The goal is to use ELISA for screening of environmental samples for estrogenic activity, and then to confirm the presence of the individual estrogens in samples that test positive with LC/MS/MS.

The research is ongoing. Preliminary results show the analysis works well for ground water samples, with ELISA detection limits on the order of .05 ng/l and 2 ng/l for estrogen separation and identification by LC/MS/MS. One problem is that ELISA often yields 17a-estradiol concentrations orders of magnitude higher than what can be confirmed by LC/MS/MS.

Pigs are not typically given growth hormones, so most of the focus with them is on the natural ones produced by the pigs themselves. Poultry are not generally given growth hormones either, although they still produce quite a bit of natural estradiol. Hutchins said there are estimates that the US poultry population produces between 160,000 and 760,000 kg/year of estrogen.

With Cattle however, an estimated 90% are fed growth hormones including estrogens, androgens, and progestins. Scientific literature on the matter revealed an average estradiol concentration of 10-13 ng/l in the urine. After administration of growth hormone, the estradiol concentration is 5-6 times higher.

Suresh Rao, a researcher at Purdue University discussed "Investigations of Sorption and Transport of Hormones and Animal Pharmaceuticals: Initial Laboratory Results." Pigs are big business in Indiana with a total population of 5 million. Although the number has remained constant, the CAFOS have become more concentrated with a reduction in the number of operations from 25,000 to 4,500 between 1979 and today. Over 60% of these operations have more than 2,000 hogs. The concern is that a large number of pharmaceutical compounds and antibiotics are used as growth promoters.

A report from the Union of Concerned Scientists claimed that the anti-microbials used in human health are dwarfed by animal uses for non-therapeu-

(Continued on next page)

E/E Conference Report

CAFOs

(Continued from page 14)

tic purposes. About 10.3 million pounds/year are used on hogs, 10.5 million pounds/year on poultry, and 3.7 million pounds/year on cows, compared to about 3 million pounds per year on humans. The animal numbers would be even higher if one were to consider therapeutic uses as well.

Rao said the initial project was to identify major growth promoters and hormones present in swine CAFOs. The major hormones included 17a-estradiol, 17a-ethinylestradiol, and testosterone. Growth promoters included carbadox, tylosin, tetracycline, and bacitracin.

Rao said that he started out thinking that birth control pills and hormone replacement therapy would be the largest sources of hormones from people. But subsequent research revealed that 60% of the excreted hormones in wastewater come from pregnant women. The total human load of estrogen is estimated at 675 kg/year. With a few bold assumptions about wastewater load, Rao estimated human estrogen excretion results in an average load of about 300 ng/l.

Another issue is the hydrological transport of these compounds. Rao said they have a high sorption coefficient and that the facilitated transport to water bodies is possible. They are completing lab experiments to continue to collect data and there are plans to conduct field experiments to measure what happens in a field that receives manure applications. There are plans to go out to the watershed in a large CAFO and monitor water from the source to various distances from it.

Helder Hakk, a researcher with the USDA's Biosciences Research Lab Animal Metabolism-Agricultural Chemicals Research Unit presented a talk on the "Fate of the Endogenous Hormones 17a-estradiol and Testosterone in Composted Poultry Manure and Their Sorption and Mobility in Loam Soil and Sand." He noted that estradiol is one of the most potent hormones and is about 1,000 to 100,000 times as potent as alkylphenols. It is naturally excreted in the urine of mammals and birds. Hakk estimated that chickens on the east shore of Maryland produce about 6 kg/day of estradiol.

A variety of effects of estradiol have been noted. At low levels (5-500 ng/l) estradiol increased alfalfa growth. At higher levels (5000-500,000 ng/l) estradiol decreased alfalfa growth. Levels of 300 ppb in poultry feed caused premature udder development. 1-10 ng/l increased vitellogenin growth in fish.

Hakk said, "Up until this point, the main concern has been nutritive, but little attention has been on the chemical nature of some of the minor materials in the manure." He decided to find out if composting could help reduce the levels of estrogen in the manure.

For an aerobic composting process, he used a 2-acre site with an 8" thick pad separating the compost from the dirt. The composting recipe they tried combined chicken manure with a variety of commercial products to create a carbon to nitrogen ration of 30:1. They were deposited in 160'x5' rows, 40" high.

Throughout the composting process, samples were quick frozen and sent out for analysis. Testosterone concentrations started at around 200 ng/g, and degraded at an average rate constant of .0752/day. The bulk of the degradation occurred early in the study when the high temperatures were most active. Estradiol concentration stated at 100 ng/g, and degraded at a rate constant of .0121/day.

Hakk believes there are two rates of degradation occurring. A rapid degradation during the thermophilic period, and then a slower degradation after the compost cools down. In the end, neither hormone was degraded completely after 19 weeks. He said, "Composting may provide an environmentally friendly method for reducing, but not eliminating the introduction of potent endocrine hormones."

Hakk also reported on the results of another study looking at the transport and fate of testosterone and 17a-estradiol in columns packed with loam soil or sand. Combustion analysis revealed that 80% of the testosterone and 96% of the estradiol remained in the top 5 cm of soil. The conclusion was

(Continued on next page)

E/E Conference Report

CAFOs

(Continued from page 15)

that estradiol and testosterone will be readily transported through the sand, but they are rapidly and strongly sorped into loam soil. Preliminary data also suggests that they may be metabolized in soil.

Future work includes confirming the compost extraction test results with mass spectrometry. Hakk would like to experiment with prolonging the thermophilic heated phase by adding extra carbonaceous material to see if that works better at reducing the hormones.

Resources:
Stephen Hutchins: 580-436-8563 / hutchins.steve@epa.gov
Suresh Rao: 765-496-6554 / pscr@purdue.edu
Heldur Hakk: 701-239-1293 / hakkh@fargo.ars.usda.gov

Wastewater Treatment

Human waste streams contain a number of hormones and hormone modulators. Greg Sayles, a chemical engineer with the US EPA's NRMRL said the most important compounds from a sewage treatment perspective are natural and synthetic hormones and alkylphenol polyethoxylate related compounds. The former are either manufactured directly by humans or consumed in the form of hormone replacement therapy and birth control pills. The latter are often used in soaps and wetting agents.

A1998 report in Environmental Science and Technology, by Desbrow et al reported that public owned treatment works discharged several estrogenic compounds including estrone (1-50 ng/l) 17 B-estradiol (2-50 ng/l) and 17 a-ethinylestradiol (0-7 ng/l)

Sayles said these chemicals are accumulating in ecosystems impacted with sewage treatment effluent, indicating that they are not being adequately degraded in the sewage treatment plant. Alkylphenol polyethoxylates have been shown to lose portions of the polyethoxylate chain during sewage treatment, however the most prevalent resultant compound, nonylphenol, is known to accumulate and cause toxicity in aquatic organisms even at low concentration. Thus degradation poses more of a risk than the parent compound. Sayles said, "There is no evidence that alkylphenols are destroyed, just transformed."

According to Sayles, over 500 million pounds of NPE surfactants are used in the US annually and about half of them end up going into the sewage system. Estradiol has been detected at levels as high as 50 ng/l in sewage. Estriol estrone has been detected at levels up to 100 ng/l.

Research indicates that some plants do better at breaking down hormones, but more research is needed to determine why. Two factors that have been identified are retention time and nitrification.

Sayles said, "We need detailed studies on the fate of these EDCs within the plant. We really need some good study on what happens within the plant and how variables like temperature affect it. We should also be thinking about other treatment systems. Most of the data we have is associated with the big plants, but a large number of people in the country use septic systems on their own land or small community treatment systems."

Paul McCauley, with the US EPA's NRMRL discussed a pilot plant to study different treatment approaches of human sewage, and the effectiveness of each step in wastewater treatment. The long-term objective is to determine the fate of estrogenic compounds and to possibly fashion a cure for the problem.

His team has built two pilot scale wastewater treatment system to model metabolic pathways to study aerobic and anaerobic digestion. It uses rabbit waste mixed with glycerin to simulate fats.

McCauley said, "Our primary objective is to get a pilot plant running and identify what works. We need some cursory comparisons to outside plants."

E/E Conference Report

The Challenges of Combustion

Combustion processes have been implicated as a source of EDCs in the air. Brian Gullet talked about "Endocrine Disruptors from Combustion and Vehicular Emissions: Identification and Source Nomination." There are ongoing efforts to analyze exhaust samples from combustion and vehicular sources to provide initial identification of EDCs. The intent of this screening effort is to provide discerning evidence for nominating sources for further EDC characterization. Conventional sampling, advanced analytical methods, and bioassays are being used to provide initial characterization of these samples for their compound identity and EDC activity.

Gullet said the intent is to sample and chemically characterize multiple combustion sources, consistent with the likelihood that combustion source EDC exposure is not linked to any one source. For example, since body burdens of polychlorinated dibenzodioxin/dibenzofuran do not appear to be elevated near traditionally suspected sources like waste incinerators, it appears that exposure sources of dioxin are ubiquitous.

Sample fractionation will be coupled with chemical characterization, quantitative structure activity relationship analysis, and bioassay testing to nominate and identify potential EDC compounds in combustion emissions. The ability to provide this early source-specific EDC identification and characterization of combustion sources will be a parallel activity with more extensive health risk analysis and exposure assessment. In this manner appropriate prioritization of EDC management options can be implemented prior to definitive health effects conclusions.

Gullet said, "We only see dioxins and furans because we look for them. Our intent was to over the next year or so look at several combustion sources and go into a fairly extensive analysis of these combustion emissions to try and understand what EDC compounds are in them."

Gullet's team has analyzed a wide range of sources over the last year and a half including domestic waste burning, diesel trucks, forest fires, fireplaces, wood stoves, wheat burning, and others. He said, "The idea was to not only sample the sources, but to also apply bioassays to see which compounds were potentially EDC active."

His team has developed a novel technique called multi dimensional gas chromatography-mass spectroscopy (MDGC-MS), which gives better results than using traditional gas chromatography-mass spectroscopy (GC-MS). Many compounds were found with MDGC that would have been difficult to detect in conventional GC-MS because of elution. For example, a methoxy alkylphenol waste compound was found to coelute with biphenyl under conventional conditions but was well separated using MDGC.

They are planning to use QSAR to look at what compounds they need to focus on. There are also plans to test more construction fires, which is a substantial concern because of the estimated 500,000 structural fires a year. Other plans call for more diesel vehicle studies. The group has built the world's only diesel vehicle with a built-in system for sampling dioxin and furan emissions.

Gullet said some of the more interesting work over the last couple of years has been on emissions of dioxins and furans from backyard domestic burning. While this may not be a very common practice, the emissions per pound are approximately 4-6 orders of magnitude (10,000 – 1 million times) higher than from a controlled incinerator facility.

Tests from forest fire emissions indicated levels of dioxins and furans 10 times higher than previously thought. Gullet said the reason for the discrepancy was that this was the first time that anyone had sampled a forest fire for furans. The EPA had traditionally relied on data based on industrial combustion of wood for the previous estimates.

Resources:

Brian Gullet:
919-541-1534 / gullet.brian@epa.gov

E/E Conference Report

Cost Effective PCB Cleanup

PCB cleanup costs can be quite substantial. For example, GE is spending $175/ cubic yard to dredge the Hudson for PCBs. A much more cost-effective option is natural recovery, in which the forces of nature hide the problem with little or no human intervention. Richard Brenner, an engineer at the EPA's NRMRL presented a talk on the effectiveness of this technique.

Monitored natural recovery includes careful assessment, modeling, and monitoring to ensure success. Processes that contribute to natural recovery include biological processes, physical and chemical processes, and sorption. Storm events, construction and industrial activities can all work against the process by disturbing the sediments.

A Clemson, SC site was selected because it had a documented history of contaminated sediments. Capacitor manufacturers dumped an estimated 400,000 tons of PCBs into the water between 1955-1978. Battelle collected core samples at 10-transect locations between 1994 and the present time.

An analysis of the results indicated that beginning in 1955, PCB levels started to increase until 1977 when operations ceased. Then PCB levels began to decrease down to about 1.5 ppm. Brenner said it is estimated it will take another 1-5 years to reduce surface concentrations below 1 ppm, and then another 10-30 years before they reach .05 ppm.

Resources:
Richard Brenner: 513-569-7657 /
brenner.richard@epa.gov

Finding EDC Alternatives

As the various efforts to identify and characterize EDCs proceed in the US, Europe, and Japan, manufacturers will eventually be faced with the prospect of finding alternatives to some of the chemicals. The danger is that some of the alternatives may be just as bad, if not worse than the original chemicals. In order to address this issue, the EPA has launched a program for the identification and replacement of EDCs.

Douglas Young with the US EPA's NRMRL said there are two distinct phases of the program involving 1) identifying potential EDCs, and 2) providing knowledge on replacing these in current commercial applications. Young said they would like to incorporate this knowledge into software that could be used by commercial and government researchers.

The EPA is looking at using a QSAR model such as COREPA and COMFA to identify compounds of concern. Young said they would like to fund someone to do a 3rd model that would be complementary to these other 2 models.

For the second part of the project, they want to develop a piece of software to suggest replacements for EDCs in anti-oxidants and surfactants applications. The EPA has already developed software called Program for Assisting the Replacement of Industrial Solvents (PARIS) for chemical solvents. Young said they would like to repurpose the coding and framework of PARIS for EDCs. However the primary properties of anti-oxidants and surfactants will be different than for solvents. A limitation of this kind of technology is that it will not work for all EDCs such as pesticides and herbicides.

The RFPs for this project are due by the end of February, with the intent of getting the project up and running by mid fall. Meanwhile, Todd Martin at the EPA is working on converting the PARIS program framework to work with EDCs. Young expects the program to be completed in about 3 years.

Resources:
Paris: http://www.tds-tds.com/parfact.htm
Douglas Young: 513-569-7624 /
young.douglas@epa.gov

Program for the Identification and Replacement of Endocrine Disrupting Chemicals

Douglas Young

US EPA

National Risk Management Research Laboratory

Cincinnati, OH

Project Outline

- Still in planning phase
- Develop a unique method for identifying potential EDC (external)
- Develop a program to aid in suggesting replacements for EDC (internal)
- If possible, combine into one software program

Identifying Potential EDC

- Use a QSAR type approach to quickly identify potential EDC (external)
- No duplication of concurrent efforts (such as COREPA and CoMFA)
- Use as another validation tool for concurrent efforts
- There is a Request for Proposals (RFP) to establish a collaborative agreement currently underway

Identifying Potential EDC cont'd

- Collaborator to be largely responsible for this portion of the project (Thesis or Dissertation work)
- We can help collect data needed to fill library

Replacement of Potential EDC

- Develop software that will aid in suggesting replacements for known or potential EDC (internal)
- Off shoot of the PARIS II Project (Program for Assisting the Replacement of Industrial Solvents)

PARIS II

- Suggests possible replacements for currently used solvents or solvent mixtures
- Uses the DIPPR (Design Institute for Physical Property Relationships) and UNIFAC to estimate 20 physical and chemical properties of the solvent(s) to be replaced
- Tries to find best match within database of over 1500 chemicals

PARIS II cont'd

- If it can't find a single replacement, it will allow the user to design a mixture
  - The user chooses the primary component and then the program will find the chemical that would form the best mixture
- If it can't find two component replacement, it will allow the user to add a 3rd chemical to the mixture
- Ad infinitum

EDC version of PARIS II

- The framework and coding are established
- Select key physical and chemical properties to satisfy the requirements for the specific EDC application

- Might not work with compounds that are designed to be pesticides & herbicides

Example

- Di(2-ethylhexyl)phthalate (DEHP)
  - Used as a plasticizer for PVC medical tubing
  - FDA has released a safety assessment on DEHP
  - Found that infants in certain circumstances may be exposed to unacceptable levels of DEHP
- Replacement outline
  - Describe qualities that are required for a plasticizer
  - Search the database for possible replacements

Example Cont'd

- Replacement Outline
  - Describe qualities that are required for a plasticizer
    - Plasticization Efficiency
    - Tensile strength
    - Vapor Pressure
    - Solubility
    - Toxicity
    - Viscosity
  - Match properties using components in database that have less potential for ED activity
  - Suggest a replacement or replacement mixture

Final Product

- A software program that will have a methodology to identify potential EDC and then will allow the user to design possible replacement chemicals (non potential EDC) based on desired properties

- Will be used as another validation tool to compliment the concurrent research projects

Timeline

- External
  - Determine collaborator during Winter of 2002
  - Project will begin Spring/Summer of 2002
  - Expected to last 3 years
- Internal
  - Post-doc already in place
  - Project began January of 2002
  - Expected to last 3 years

Region 5 Endocrine Disruptor (ED) Efforts

Peter Howe (WD)

Lawrence Zintek (CRL)

Dennis Wesolowski (CRL)

Al Alwan (WD)

John Dorkin (WD)

George Azevedo (WD)

Mari Nord (CRL)

Marc Tuchman (GLNPO)

Babu Paruchuri (CRL)

Involvement

Division Directors
Jodi Traub
Gary Gulizean
Norm Niedergang
Bob Springer

Region 3
Jennifer Gundersen
Ron Landy

OPPT
Don Rodier
Dave DiFiore
John Walker

Water Division
Toxics Reduction Team
Central Regional Laboratory
GLNPO
Office of Regional Council

USGS
John Gannon
Steve Smith

ORD
Myriam Medina-Vera

USDA
Cliff Rice

Environment Canada
Donald Bennie

USGS
Larry Barber

Are These Compounds Present in the Region at Effect Levels Reported in the Literature?

1. Alkylphenols- NP, NP1EO, NP2EO, NP1EC, NP2EC and Octylphenol
   -Degradation Products of Nonionic Surfactants
   -Attributed to Fish Endocrine Disruption
2. Sex Hormones (Estrogens/Androgens)
   -Natural and Synthetic

Why is Region 5 Involved?

- Researchers Have Shown APES to Exist at Levels of Concern
- These Chemicals Cause Endocrine Disruption in Fish
- Needed Analytical Standards
- Needed a Standard Method That States and Environmental Labs Could Use

This Presentation

1. Alkylphenol Background
2. Region 5 Central Regional Laboratory Methods Initiative
3. Region 5 Water Division Studies
   - A. Sediment
     - -Chicago River
     - -DuPage River
     - -Des Plaines River
     - -Fox River
   - B. Water (New Data)
     - -Chicago River
     - -Calumet-Sag Channel
     - -Lower Des Plaines River
     - -Illinois River

This Presentation

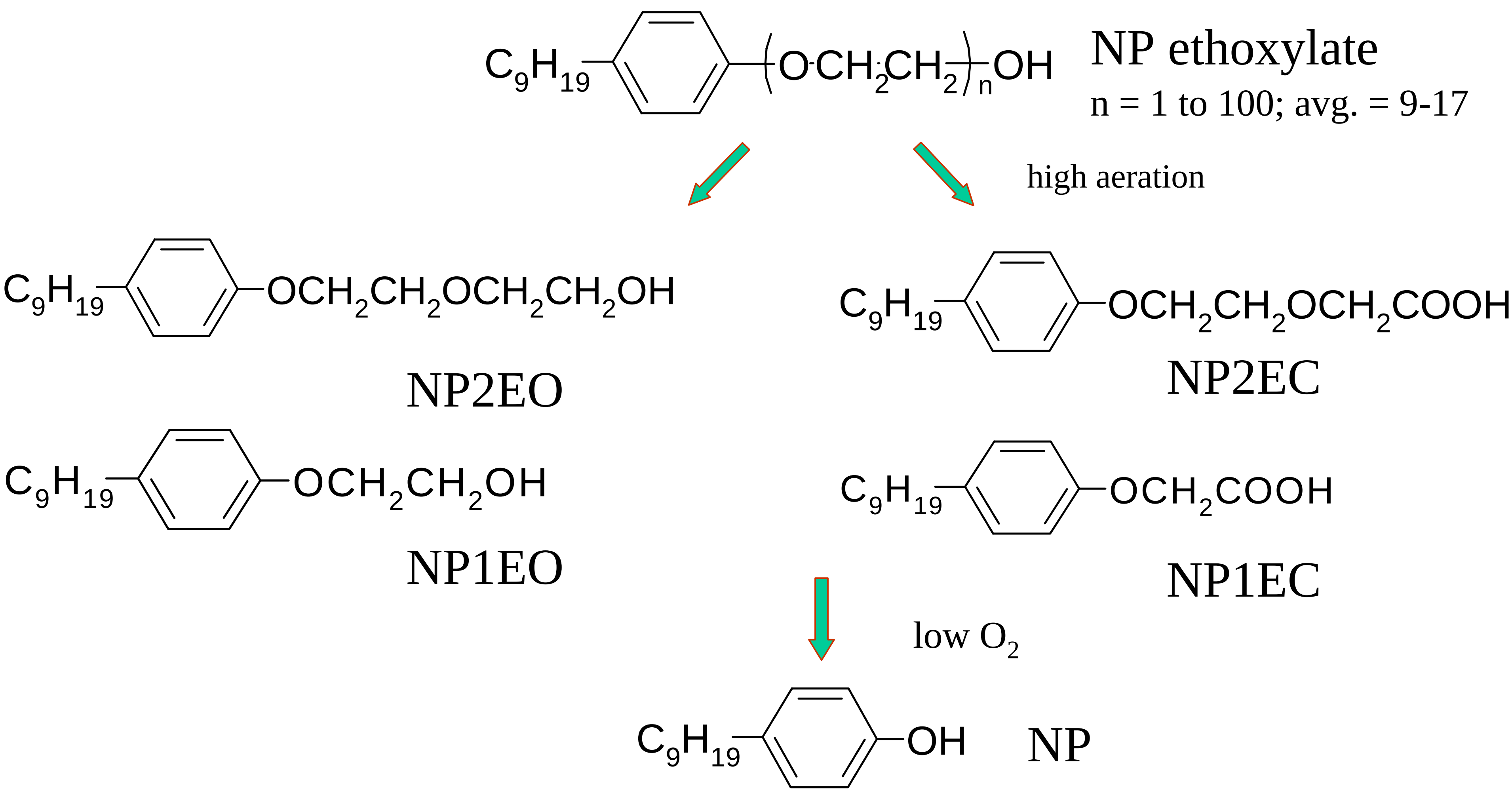
4. GLNPO-USDA Ongoing Fish Study
5. USGS Work in Progress/Future Work
6. Region 5 Work in Progress/Future Work
7. Conclusions

Alkylphenol Compounds
How Are They Used ?

- Industrial, Institutional and Domestic Surfactants
- Antioxidant in Plastics
- PVC Stabilizer
- Oil Additives
- Oil Field Recovery
- Metal Extractants

What Happens When Nonylphenol Ethoxylates Are Released Into The Environment ?

Common Environmental Metabolites of Nonylphenol Ethoxylates (Adapted from Ahel *et al*., 1994; Naylor, 1992)



Bioconcentration Factors (BCF) for Nonylphenol

Species	BCF	Reference
Fathead Minnow	271-984	Ward and Boeri (1991a) Brooke (1993)
Three-Spined Stickleback	1300	Ekelund et al.,(1990)
Mussel	14	McCleese et al., (1980)
Mussel	3400	Ekelund et al., (1990)

The Elusive Bottom Line On Endocrine Disrupting Effects

- Fathead Minnows
  - Stimulated Production of Female Hormone at 50-100 ppt
  - Levels of NP Greater Than 3.4 ppb Ceased Egg Production (Giesy *et al*., 2000)
  - NP at 1.6 ppb had an Adverse Effect on Sertoli Cells (Miles-Richardson *et al*., 1999)

The Contribution of Ammonia, Metals and Nonpolar Organic Compounds to the Toxicity of Sediment Interstitial Water from an Illinois River Tributary (Schubauer-Berigan and Ankley, 1991)

* TIE conducted on the pore water from Calumet Sag Channel sediments indicate that nonylphenols are attributed to the toxicity of the sediments to Ceridaphnia dubia, a small crustacea that lives in the interface of the water and sediment that is a marker to indicate toxicity depending on its reproduction and mortality.

World Regulatory Activities

- APE Bans, Phase outs or Use Restrictions
  - Denmark
  - Japan
  - Germany
  - United Kingdom
  - Belgium
  - Switzerland
  - Chile
  - Sweden
  - Netherlands

Recent Regulatory Activities

- Environment Canada Draft WQC = 1 & 0.7 ppb NP for Freshwater and Saltwater, Respectively
- EU Completed Their Risk Assessment and Recommended Banning "Down the Drain" Uses
- Japan Draft WQC = 0.6 ppb

Relevant Nonylphenol Concentrations

Concentration (PPB)	Effect/Criterion
6 ppb- Fresh Water 1 ppb- Salt Water	Draft U.S. WQC
2	Lowest Effect Concentration in Multigenerational Fish Study
1	Draft Canada WQC
0.05-0.10	Stimulation of E2, Egg Production, VTG

Documented Range of Alkylphenol Concentrations in Large Region 5 Sewage Treatment Plant Effluents (ppb)

(Larry Barber, USGS Survey)

Compound	Chicago Area	Minnesota	Detroit
NP	1.4-1.7	0.9-2.1	19
NP1EO	4.1-13	5.8-12	55
NP2EO	3.7-7	0.78-19	110
NP3EO	ND	ND-2	17
NP1EC	16-29	21-60	34
NP2EC	44-92	56-100	70
NP3EC	4.1-9.5	7.8-13	4.6
NP4EC	1.3-6.1	2.3-6.5	1.8

Concentrations are Above Effect Level for ED Based on the Literature

Region 5 CRL Methods Initiative

- CRL Director (Dennis Wesolowski) Developed Methods Initiative Proposal for 40 CFR 136 Incorporation, a Cooperative Effort with ORD Cincinnati and Region 3
  - Completed Sediment SOP for NP, NP1EO, NP2EO, Octylphenol and Bisphenol A Analysis by (GC/MS/Full Scan), March 2001
  - Completed Water SOP for NP, NP1EO, NP2EO, OP and Bisphenol A Analysis by (GC/MS/SIM), September 2001
  - Completed Synthesis and Commercial Availability of Standards with Known Purity

Region 5 Central Regional Laboratory Contribution

<u>Completed Sediment Method Detection Limits (ppb)</u>

• Nonylphenol	110
• NP1EO	218
• NP2EO	433
• Octylphenol	24
• Bisphenol A	22

<u>Completed Water Method Detection Limits (ppt)</u>

•Nonylphenol	61 (Below Effects Criterion Levels)
•NP1EO	93
•NP2EO	265
•Octylphenol	10
•Bisphenol A	25

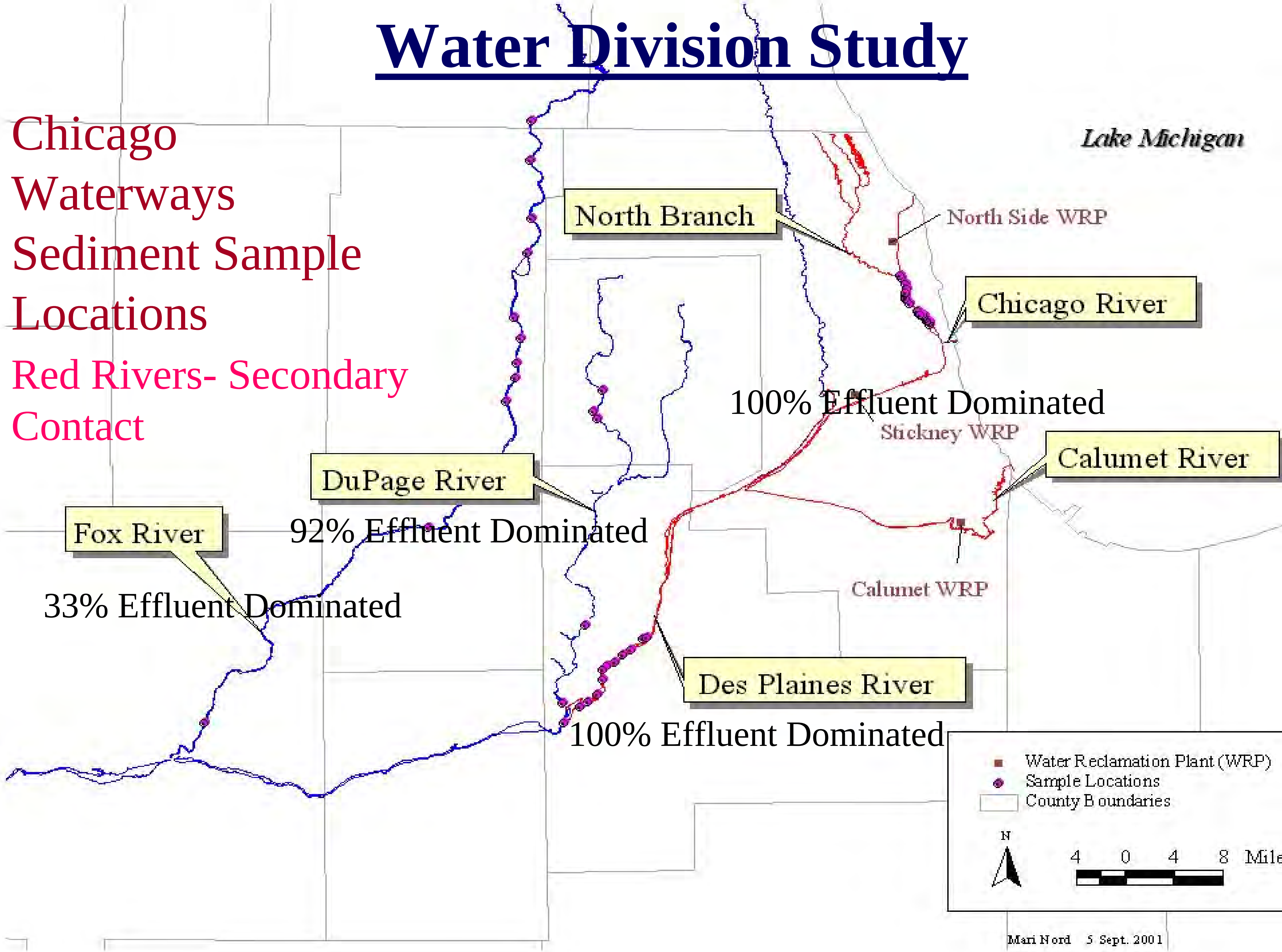
Commercially Available Standards and Surrogates

Compound	Source: Catalogue Number
NP	Aldrich: 29,085-8
n-NP	Cambridge : ULM-4559
NP1EO	Aldrich: Q2268-5
n-NP1EO	Cambridge: ULM-4520
NP2EO	Aldrich: Q2044-5
n-NP2EO	Cambridge: ULM-4521
NP1EC	Cambridge: ULM-1688
NP2EC	Aldrich: Q2109-3
n-NP2EC	Cambridge: ULM-4690

Water Division Study

Chicago Waterways Sediment Sample Locations

Red Rivers- Secondary Contact



North Branch
Sediment Grab Samples Ranges of Alkylphenols in ppm

Compound	Range in ppm
Octylphenol	0.1-1.2
Nonylphenol	2.5-48
Nonylphenol Monoethoxylate	ND-49
Nonylphenol Diethoxylate	ND-16

Lower Des Plaines River

Top Strata of Core Samples Range of Nonylphenol in ppm

Compound	**Range in ppm**
Nonylphenol	0.32-13.7

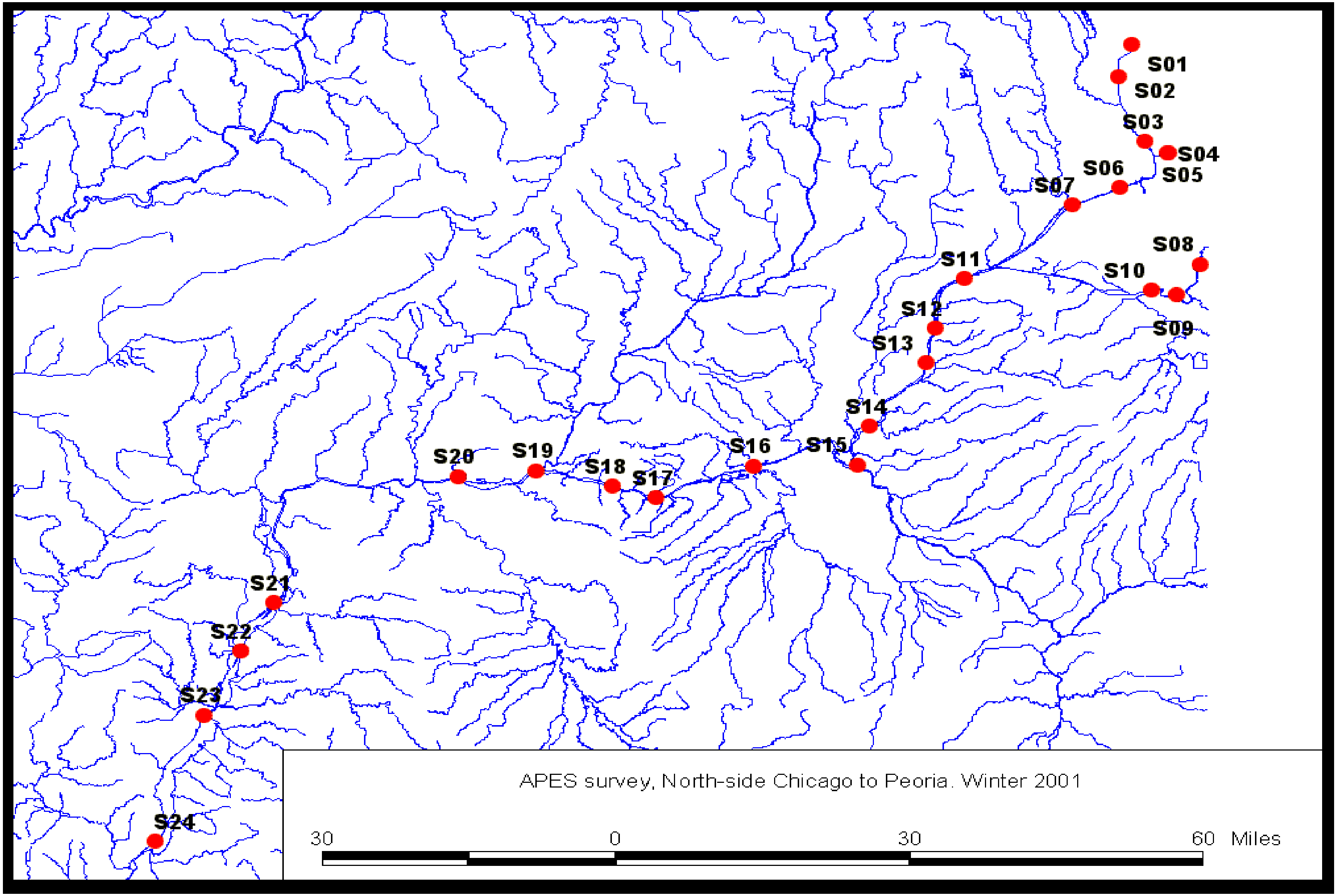
DuPage River Composite Sediment Dam Samples Ranges of Alkylphenols in ppm

Compound	**Range in ppm**
Octylphenol	ND-0.39
Nonylphenol	ND-2.5

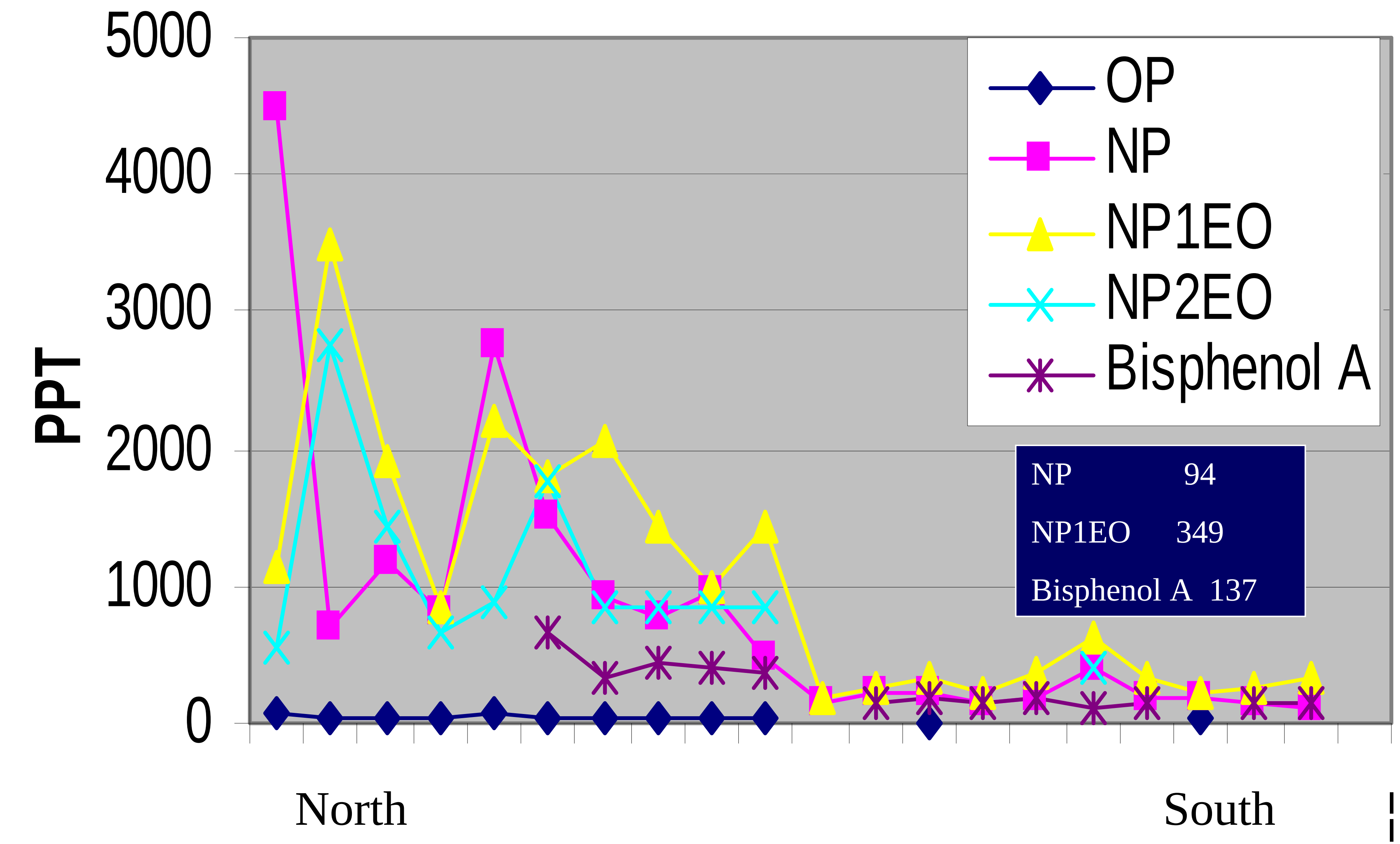
Fox River Sediment Samples

- Many Samples Contain NP and OP but All Below Reporting Limit.

Chicago Area to Peoria River Water Survey



Chicago Area to Peoria River Water Study



GLNPO-USDA
Ongoing Fish Study

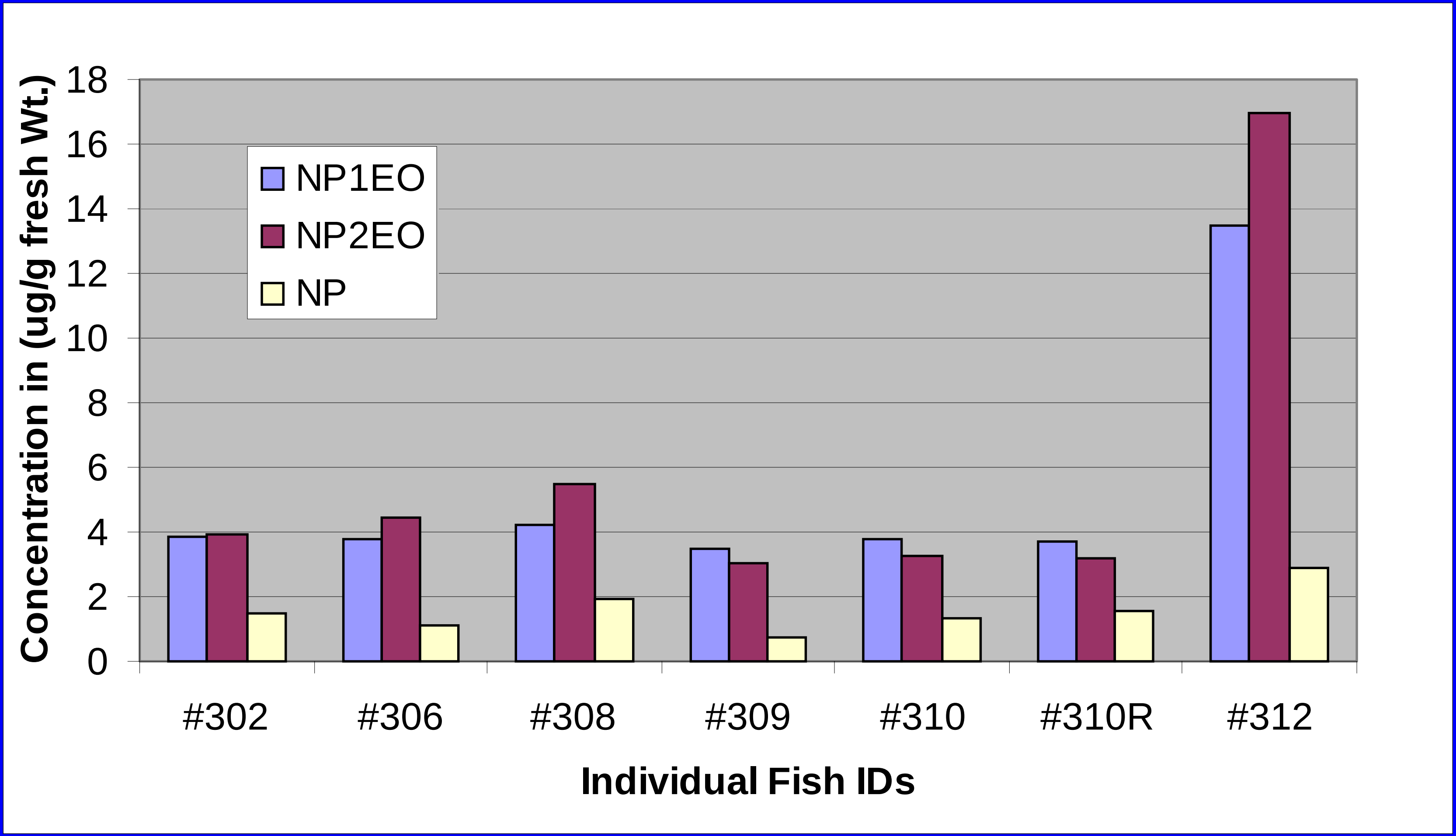
- Alkylphenols, Alkyl Ethoxylates and Their Metabolites as Potential Great Lakes, Tributaries and Effluent Dominated Stream Endocrine Disruptors. (Cliff Rice, USDA)

North Branch – Chicago River
Fish Collection/Analysis Information

- Collected by Electroshocking at RM 330-Diversey
- Analyzed for Nonylphenol-APEs (0-5 ethoxy-substituted) by LC/Fluorescence with Purified Standards
- Confirmation by GC/MS/NCI and LC/MS

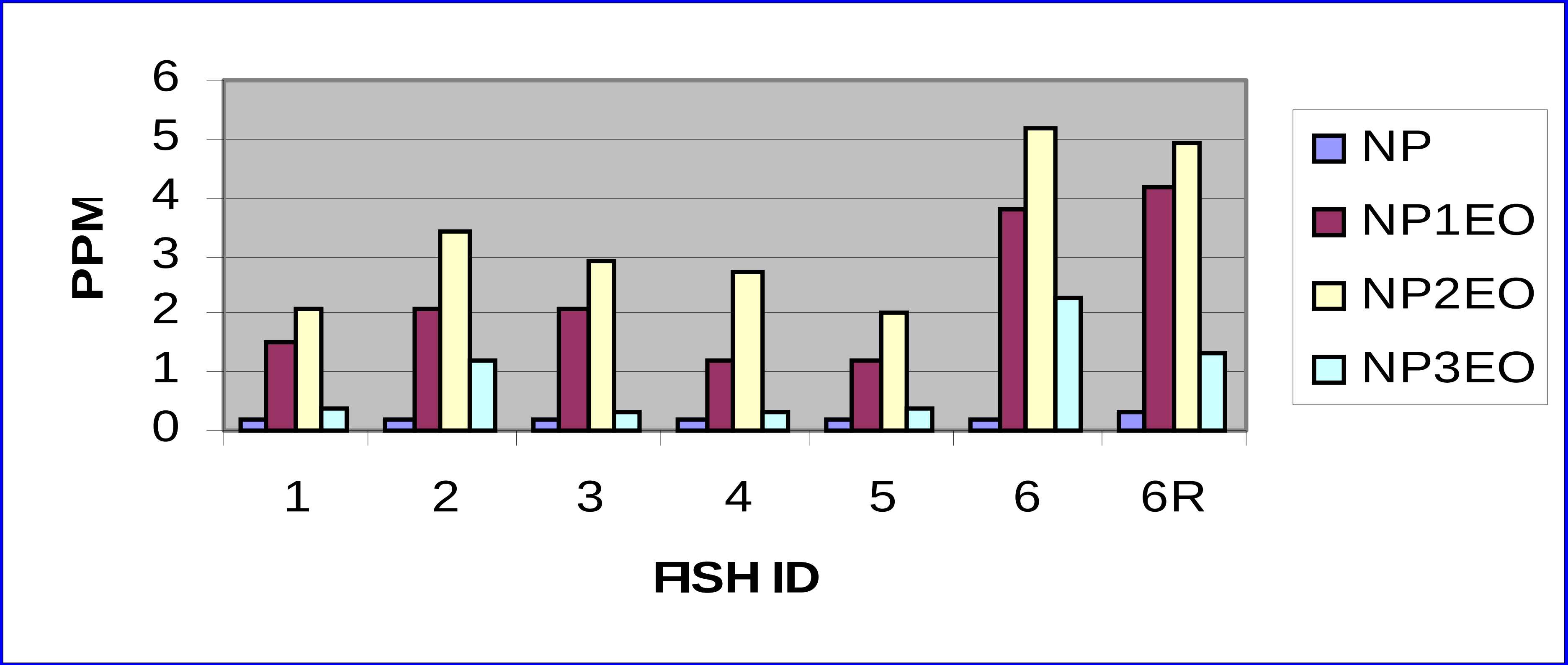
Preliminary Results for Total Alkylphenols (NP, NP1EO and NP2EO) in Carp North Branch - September 1, 1999

(Clifford Rice, USDA, Beltsville, MD)



St. Paul Minnesota Metro POTW Walleye Alkylphenol Concentrations in PPM

(Clifford Rice, USDA, Beltsville, MD)



Nonylphenol Half Life in Muscle and Fat of Rainbow Trout ~19 Hours (Lewis and Lech, 1996)

GLNPO-USDA Fish Study
(Cliff Rice, USDA)

- Lower Des Plaines River
  - Total NPEs (0-3) 1.55 ppm
  - Abnormal Left Gill
  - Masses on Gonads

- North Branch Chicago River- Fish #312
  - Total NPEs (0-3) 33.3 ppm
  - Tumerous Growths on Ovaries , One Large and One Small
  - Large Spleen

Work in Progress
GLNPO-USDA Fish Study

- Serum Testosterone, Estrogen and Vitellogenin Levels and Evaluating Gonad Histopathy for Fish
- Analyzing Fish Tissue for Polybrominated Diphenol Ethers (Flame Retardants) Which Appear to be an Emerging Issue

Work in Progress/Future Work

Proposed Study from the USGS
Minneapolis, MN – METRO POTW
Larry Barber and Kathy Lee

- Fathead Minnow Study

  Toxicity Identification Evaluation (TIE)
  - Serum VTG
  - Testosterone/Estrogen
  - Gonad Histopathy

Hormone Work in Progress
(Larry Barber et al., USGS)

- Investigation of the Fate of Natural and Synthetic Sex Hormones in Sewage Treatment Plant Effluents
- Determine Fate in Receiving Stream
- Conduct TIE (Toxicity Identification Evaluation) to Determine Major Contributors to Fish ED

Effects of Exposure to 17 β-Estradiol to Fathead Minnows

(Miles-Richardson et al., 1999)

- Threshold for Histologic Changes in Testes ~0.04 ppt
- Threshold for Vitellogenin Induction ~0.04 ppt

β-Estradiol Effluent Concentrations at Four Michigan STPs
(Snyder et al., 1999)

- Average Concentration= 1.5 ppt
  (Range= ND to 3.7 ppt)

Sewage Treatment Effluent Concentrations 10-30 Fold Higher than LOEC for Endocrine Disruption

Others- Including Ethynyl Estradiol

Region 5 Work in Progress/Future Work

- Improve Techniques/Methodologies
  - Solid Phase Extraction
  - GC Columns/Conditions/Derivatization
  - Other Options
- Sediment and Water Monitoring
- Investigate Other Compounds of Interest
  - NP1EC and NP2EC
  - Hormones and Pharmaceuticals

Conclusions

- NP, NP1EO, NP2EO and OP in Water Column and Sediment
- Need to Include NP1EO and NP2EO with NP to Assess Total Exposure To Biota
- Sediments are Important Reservoirs
- Must Work on Exact Chemistry of Toxic Component and Not Generalize to Mixtures

Conclusions

- APEs are Present in the Water Column from Chicago to Peoria (over 100 miles studied)
  - Detected at Lower Concentrations Downstream due to Dilution, Deposition and/or Decomposition
- Standard Methods Enable Others to Sample/Gather Information to Determine APES Concentrations Nationwide
- Drives Positive Environmental Outcomes
  - Enhance Leverage for Voluntary Reduction
  - Enables Regulation and Develop Potential Banning
  - Enables Setting of Permit Requirements