Defining the Role of Pollutants in the Disruption of Reproduction in Wildlife

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Although chemical exposure has been associated with reduced reproduction in certain North American fish, reptiles, and mammals, definitive cause-and-effect data are lacking in many instances. Because the increasing use and global transport of industrial chemicals pose significant risk to successful reproduction, methods should be developed that can define the geographic extent and magnitude of injury and risk to wildlife. Because industrial chemicals are articles of commerce, information about injury to wildlife has been contentious and too often ineffective in changing societal behavior. The following strategies are advocated for inferring causal relationships. First, a balanced and comprehensive assessment of the data is necessary to determine the geographic extent of exposure and reproductive effects associated with environmental pollution. Initial efforts to document reproductive injury should focus on specific ecosystems in which detrimental effects have been observed, but lack sufficient causal data. Model systems (including experimental mesocosms or field ecosystems) should be identified or designed that can adequately test multigenerational reproductive effects. Mechanistic data from supportive laboratory studies on reproductive toxicity, quantitative structure-activity relationships, and bioaccumulation can be used to predict effects of related pollutants and to determine risk. Such information is essential to prevent future injury to wildlife and to prioritize the numerous remediation decisions facing our society. — Environ Health Perspect 103(Suppl 4):87-91 (1995)

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Introduction

In the last 50 years, increasing production and use of industrial chemicals has led to the worldwide contamination of ground water, lakes, and oceans (1). Contaminants can enter the food chain and some can subsequently bioaccumulate in wildlife. A few of these chemicals have been associated with reproductive injury in wildlife (2-7). In December 1993, a group of North American wildlife biologists convened to critically assess evidence of specific instances where these contaminants were reported to produce deleterious effects in wildlife populations through interference with reproduction or embryonic development. Because of the large historical database on the Laurentian Great Lakes, the majority of the studies focused on this area, although reports were also presented on lakes in Florida, coastal Florida, Arkansas, New York, the western coast of the United States, the Saint Lawrence River, and British Columbia, Canada. These reports are presented elsewhere in this volume.

The goals of this conference were 2-fold: to determine the geographic scope and magnitude of reproductive and developmental effects in wildlife on the North American continent, and to determine any commonality of the causes or mechanisms accounting for the observed changes. The participants in the conference concluded that:

- Throughout North America, there is widespread exposure to environmental chemicals capable of disrupting development and the reproductive, nervous, immune, and endocrine systems in animals (8).
- Many of these contaminants mimic or inhibit hormones, thereby modifying development and reproduction. There is convincing evidence for detrimental effects of these contaminants in certain wildlife populations of North America (1).
- However, we are uncertain of the geographic extent to which contaminants contribute to the degradation of wildlife populations, and data from mechanistic studies must be available before detrimental effects can be predicted.

This exercise convinced us of several points regarding our role as scientists in our rapidly changing world. Most important, it is our responsibility to provide the best available information to environmental managers even in the face of incomplete answers. Over a thousand chemicals are introduced annually into the environment (9), and complete toxicity information on every chemical for the exposed wildlife will never exist. Thus, despite testing and precautions, wildlife populations are at risk of injury.

One approach that might be useful when injury occurs is the ecopidemiological inference method outlined by Fox (9) that is based upon traditional human epidemiology. The ecopidemiological approach also has value for weighing evidence to determine if the relative risk warrants policy changes. Another strength of the ecopidemiological method is its predictive power. For example, knowledge of mechanisms of actions can allow the prediction of biological effects of related compounds (10,11) and more accurate risk assessment (12). Such inferential information is extremely powerful for predicting and preventing detrimental effects on wildlife and frequently essential for regulatory decisions.

Recommendations for Environmental Risk Assessment

It is now essential to develop methodologies to assess to what degree endocrine-disrupting chemicals contribute to the continental and global population decreases currently reported. For example, a number of studies have clearly shown that wildlife
exposed to various estrogenic contaminants under laboratory conditions exhibit symptoms mimicking those of wildlife living in close association with major contaminant sites (1). Theoretically, endocrine-disrupting contaminants have the potential to cause catastrophic declines in global wildlife populations. At specific localities, these compounds have caused massive decreases in wildlife populations and have apparently inhibited the recovery of many populations due to their multigenerational effects (1). However, the global influence of these compounds must be determined. Our working group (which also included Charles Peterson, Malcolm Ramsay, and Donald White) proposed several specific areas of study that would define the role of pollutants in causing reproductive injury to wildlife and improve our predictive abilities for environmental risk assessment.

**Recommendation One**

A balanced and comprehensive assessment of the data should be conducted linking exposure and reproductive injury to wildlife on a continental and global basis. Documentation and critical assessment of observational associations between contaminants and wildlife declines are essential because toxicologists, ecologists, and resource managers infrequently interact, and there appears to be a general reluctance among resource managers to consider that toxic chemicals can effectively reduce exposed populations (13–15). Most field studies yield associations between reproductive injury and chemical contaminants, not definitive cause-and-effect relationships. The evidence for causality of reproductive injury can be systematically examined using the ecopidemiological criteria proposed by Fox (9). For an individual data set, this approach provides a measure of the association’s probability, chronological relationship with exposure, strength of the association, and specificity (9). Information from different data sets can be synthesized to evaluate other criteria of causal inference (consistency on replication, predictive performance, and biological coherence).

At present, a comprehensive synthesis of the various data sets linking exposure and wildlife effects has been performed on only one geographic region, the Laurentian Great Lakes. The Fox criteria (9) were applied at the First Cause–Effect Linkages Workshop in 1989. A subsequent study, "Great Lakes—Great Legacy," drew on those data and other work to demonstrate the extent of the injury (16). The first Wingspread Conference in 1991 clearly showed that sublethal, multigenerational effects of endocrine-disrupting contaminants were responsible for a significant decline in many of the vertebrate (fish, bird, mammal) populations in and around the Laurentian Great Lakes (1). This type of synthesis is essential to direct future scientific research and modify environmental policy. If we are to determine the effects on other populations throughout the North American continent, and in fact worldwide, we need a comprehensive database.

One of the greatest strengths of this synthesis would be an evaluation of consistency among individual data sets involving different geographic areas, times, populations, investigators, and research designs and would be the basis for decisions using the weight of evidence approach. This information could be incorporated into the Geographic Information System (GIS) format and provide mapping of historical, current, and emerging pollution sources as well as effects data. A GIS format would facilitate modelling of transboundary pollutant flow, and information could be linked to other global processes such as ozone depletion and habitat destruction. Implicit in this approach is the need for an institutionalized, long-term funding commitment.

**Recommendation Two**

Model ecosystems should be developed to define the detrimental effects of endocrine-disrupting contaminants in the field and to establish causal linkages between contaminant effects and individuals, populations, and communities. As discussed above, the majority of the currently available data on the detrimental effects of endocrine-disrupting contaminants on North American wildlife comes from studies performed in or around the Great Lakes or other temperate aquatic ecosystems such as the Chesapeake Bay and the western coast of the United States. More recent studies have begun to focus on other aquatic ecosystems, such as the warm, subtropical, shallow lakes of central and South Florida (17, 18). The emphasis on aquatic ecosystems is primarily due to the fact that many of the chemicals that affect reproduction are lipid-soluble, bioaccumulate through the aquatic food chain, and frequently exert effects in highly visible species at the top of the food chain (1, 8, 16). Obviously, a continental or global risk assessment of these contaminants to wildlife should also include data from currently underrepresented ecosystems (e.g., terrestrial ecosystems such as temperate and tropical wetlands, deserts, and high elevation communities) and from organisms on all trophic levels. Currently, freshwater fish and birds feeding in lakes are the most extensively studied organisms (1). Although global population declines are under way for many amphibian (19–21) and chondrichthian (22, 23) species, no published studies are available on the biological effects of sublethal doses of chemicals with known endocrine-disrupting or reproductive activities in these species. Could these declines be attributed, in part, to the deleterious effects of such contaminants?

Until the recent meeting organized by the World Wildlife Fund, few opportunities have existed for biologists from varying disciplines to discuss their concerns and data on this topic with a continental or global perspective. A clear consensus from the meeting called for future research to define model systems that could be used to document multigenerational reproductive effects. These model systems could be controlled mesocosms that are representative of an ecosystem of interest or dedicated field study areas such as the Great Lakes watershed (24–26). Properties of the ideal model must first be identified by an interdisciplinary team interested in evaluating a particular system. These properties will probably be unique to each system, target species, or contaminant of concern. Initially, the most useful model systems are expected to be field study areas in which detrimental effects have been observed at the organism or population levels, but which lack sufficient cause-and-effect support.

For example, many of the highly contaminated sites identified throughout North America (e.g., U.S. Environmental Protection Agency’s Federal Priority Listing of Superfund Sites) are frequented by both local and migratory wildlife populations and have been studied by various university or governmental agency-based scientists. The majority of these studies, however, have been limited due to funding and technical expertise as they were conducted by individuals or single agencies. Identification of some of these sites as model ecosystems could rapidly stimulate interdisciplinary approaches that would yield the data needed not only to document wildlife injury but also to construct predictive models and ultimately stimulate policy changes.

Interestingly, this approach may yield the most rapid data gain, as ecological studies usually require years of intensive research before patterns emerge. Many of these ecological studies have already been
performed, but a complete interpretation of the results and assessment of risk is lacking due to paucity of causal or mechanistic data. For example, laboratory-based causal data were rapidly obtained once ecological studies identified that the population collapse of several avian species was due to eggshell thinning (27). Thus, a more rigorous demonstration of cause and effect could be initially reserved for a selected number of contaminants or chemical classes thought to have the most serious consequences based on ecological data. Likewise, these causal studies would be linked with research on the physiological (endocrine) mechanisms associated with embryonic development, reproduction, immune system function, growth and metabolism.

Using the combined data sources established above, identified ecosystems and species could rapidly be assessed. Through the use of model systems, effects at the individual, population, and community levels could be linked and relative risks calculated as the contaminant(s) flows throughout the trophic system. Because environmental contaminants do not exist singly, further studies should concentrate on evaluating contaminant synergism. The interactive effects of contaminant mixtures can be evaluated using a number of experimental designs. For example, full factorial design experiments have been shown to be powerful in discriminating individual as well as interactive effects (28,29). Studies of the interactive nature of complex contaminant mixtures may also be done in a small number of field verifications of data from laboratory bioassays, where much progress has been made to simplify our understanding of contaminant interactions (30,31).

**Recommendation Three**

An improved scientific basis for risk analysis and prediction of reproductive toxicity should be developed. This may be most effectively achieved by integrating assays of common toxic mechanisms for reproductive injury, quantitative structure–activity relationships (QSARs), and contaminant bioavailability/bioaccumulation. Environmental risk analysis and prediction of deleterious effects on wildlife should be based on an integrated set of data, including laboratory bioassays and quantitative modeling for determination of dose effect where it is applicable. Because many of the chemicals of concern affect development or mimic/inhibit hormones (8), systematic testing of environmental contaminants must include standard development assays as well as more innovative assays evaluating hormonal disruption in target species. Multigenerational testing of contaminants is also a priority, although few such assays are currently available for wildlife.

Recent progress has been made in quantifying the effects of contaminants and complex mixtures on wildlife using embryo development assays and in vitro tests. Validated mortality/teratogenicity tests are available which employ chicken (32), fish (33), and amphibian (34) embryos as well as a number of freshwater and marine invertebrates (33,35). Reproduction bioassays have also been developed for a number of freshwater and marine plants (33,35). Data from the frog embryo test (FETAX) (34) are now being used to predict the interactive effects of narcotic chemicals, which provide the simplest model of additive mixture effects (30,31). Assessment of complex mixtures with common toxic mechanisms could be achieved using assays such as the rat hepatoma H4IIE bioassay system (36–38), which is extremely promising for the evaluation of dioxinlike toxicity and yields toxic equivalency factors (TCDD-equivalents) (11) essential for modelling. Calibration of effects from egg injection studies (39,40), laboratory exposures (41), and H4IE bioassays will facilitate risk assessment of environmental samples containing polyhalogenated hydrocarbons. Similarly, an in vitro approach has yielded information on the estrogenic equivalency of environmental chemicals (42) and can be used with target wildlife species (43).

Ultimately, this information might be integrated into quantitative structure–activity relationships (QSARs) capable of predicting toxicity/hormone activity of untested compounds based on chemical structure, much the same way that carcinogenicity and mutagenicity of polycyclic aromatic compounds can be predicted (10). However, abiotic exposure measurements (such as soil or water) do not directly translate into biotic effects because contaminant bioavailability and bioaccumulation are often species- and site-specific (44). Obviously, exposure can be more accurately estimated by measurement of tissue concentrations or other measures of bioaccumulation in target organisms. For example, differential trophic distribution of contaminants through the food chain is of great importance for persistent, hydrophobic compounds where their association with suspended sediment determines entry into biota (44). Although at first appearance this may seem to complicate the prediction of toxicity to the point of impossibility, application of these approaches to specific model ecosystems might yield sufficient information to develop workable predictive assessment tools.

There is currently enormous societal pressure to identify, curtail, and clean up contaminated sites, be they chemical or nuclear contaminants. Given the limited financial resources available, the great number of these sites demands prioritizing them for remediation. As scientists, we must provide guidance for these decisions that involve huge costs and dramatic societal changes. On a global scale, we can intervene in many cases to prevent further pollution of developing countries and undeveloped regions. Scientific decision making should be shifted from being solely based upon data to an integration of data, our professional experience, and practical inference. It is difficult to advocate action in the face of scientific uncertainty, but too often we minimize the power of the information we already possess. It is time to broaden our role as scientists to develop a rationale for making urgent environmental decisions through improved approaches to investigating injury caused by contaminants, risk analysis, and predictive capability.

**REFERENCES**

1. Colborn T, Clement C, eds. Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton, NJ:Princeton Scientific Publishing, 1992.
2. Mac MJ, Edsall CC. Environmental contaminants and the reproductive success of lake trout in the Great Lakes: an epidemiological approach. J Toxicol Environ Health 33:375–394 (1991).
3. Colborn T. Epidemiology of Great Lakes bald eagles. J Toxicol Environ Health 33:395–434 (1991).
4. Gilbertson M, Kubiak T, Ludwig J, Fox G. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarities to chick edema disease. J Toxicol Environ Health 33:455–520 (1991).
5. Bishop CA, Brooks RJ, Carey JH, Ng P, Norstrom RJ, Lean DRS. The case for a cause-effect linkage between environmental contamination and development in eggs of the common snapping turtle (Chelydra serpentina) from Ontario, Canada. J Toxicol Environ Health 33:521–548 (1991).

6. Wren CA. Cause-effect linkages between chemicals and population of mink (Mustela vison) and otter (Lutra canadensis) in the Great Lakes Basin. J Toxicol Environ Health 33:549–586 (1991).

7. Hose JE, Cross JN, Smith SG, Diehl D. Reproductive impairment in fish inhabiting contaminated coastal waters off Southern California. Environ Pollut 57:139–148 (1989).

8. Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378–384 (1993).

9. Fox GA. Practical causal inference for ecotoxicologists. J Toxicol Environ Health 33:359–373 (1991).

10. Jerina DM, Lehr RE, Yagi H, Hernandez O, Dansette PM, Wielocki FG, Wood AW, Chang RL, Levin W, Conney AH. Mutagenicity of benzo[a]pyrene derivatives and the description of a quantum mechanical model which predicts the ease of carbonium ion formation from diol epoxides. In: In Vitro Metabolic Activation in Mutagenesis Testing (DeSerres FJ, Fouts JR, Bend JR, Philpot RM, eds). Amsterdam:Elsevier, 1976:159–177.

11. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 21:51–88 (1990).

12. Bretthauer EW. EPA’s approach to environmental research in the 90s. Environ Toxicol Chem 12:1331–1333 (1993).

13. Gilbertson M. PCB and dioxin research and implications for fisheries research and resource management. Can J Fish Aquat Sci 50(5):1078–1080 (1993).

14. Munkittrick KR. Ecotoxicology: cause and effect or just because. Can J Fish Aquat Sci 50:1568–1570 (1993).

15. Gilbertson M. Show cause: response to Munkittrick. Can J Fish Aquat Sci 50:1570–1573 (1993).

16. Colborn TE, Davidson A, Green SN, Hodge RA, Jackson CI, Liroff RA. Great Lakes—Great Legacy? Washington: Conservation Foundation and Ottawa:Institute for Research on Public Policy, 1990.

17. Woodward AR, Jennings ML, Percival HF, Moore CT. Low clutch viability of American alligators on Lake Apopka. Fla Sci 56:52–63 (1993).

18. Guillelette LJ Jr, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 102:680–688 (1994).

19. Blaustein AR, Wake DB. Declining amphibian populations: a global phenomenon? Trends Ecol Evol 5:203–204 (1990).

20. Dunson WA, Wyman RL, Corbett ES. A symposium on amphibian declines and habitat acidification. J Herpetol 26:349–352 (1992).

21. Sherman CK, Morton ML. Population declines of Yosemite toads in the Eastern Sierra Nevada of California. J Herpetol 27:186–198 (1993).

22. Compagno LJV. Shark exploitation and conservation. In: Elasmobranchs As Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fishery. Natl Oceanic Atmos Adm Tech. Report NMFS 90, 1990;391–414.

23. Musick JA, Branstetter S, Colvoceres JA. Trends in shark abundance from 1974–1991 for the Chesapeake Bight region of the U.S. mid-Atlantic coast. Natl Oceanic Atmos Adm Tech Report NMFS 115, 1993.

24. Ernst W, Doe K, Jonah P, Young J, Julien G, Hennigar P. The toxicity of chlorothalonil to aquatic fauna and the impact of its operational use on a pond ecosystem. Arch Environ Contam Toxicol 21:1–9 (1991).

25. Axelsson B, Norgren L. Parasite frequency and liver anomalies in three-spined stickleback, Gasterosteus aculeatus (L.), after long-term exposure to pulp mill effluents in marine mesocosms. Arch Environ Contam Toxicol 21:505–513 (1991).

26. Evans MS, Noguchi GE, Rice CP. The biomagnification of polychlorinated biphenyls, toxaphene, and DDT compounds in a Lake Michigan offshore fish web. Arch Environ Contam Toxicol 20:87–93 (1991).

27. Peakall DB. DDE: its presence in peregrine falcon eggs in 1948. Science 1983:673–674 (1974).

28. Boyd CA, Weiler MH, Porter WP. Behavioral and neurological changes associated with chronic exposure to low-level concentrations of pesticide mixtures. J Toxicol Environ Health 40:15–34 (1993).

29. Porter WP, Green SM, Debink NL, Carlson L. Groundwater pesticides: interactive effects of low concentration of the carboxates, malicarb and methomyl and the triazine metribuzin on thyroid and somatotropin levels in white rats. J Toxicol Environ Health 40:15–34 (1993).

30. Shirazi MA Dawson DA. Developmental malformation of frog embryos: an analysis of teratogenicity of chemical mixtures. Arch Environ Contam Toxicol 21:177–182 (1991).

31. Shirazi MA, Linder G. A model of additive effects of mixtures of narcotic chemicals. Arch Environ Contam Toxicol 21:183–189 (1991).

32. Gebhardt DOE. The use of the chick embryo in applied teratology. In: Advances in Teratology, Vol 5 (Woolfam DHM, ed). New York:Academic Press, 1972;97–111.

33. Horning WB II, Weber CL. Short-term methods for estimating the acute and chronic toxicity of dioxin-related compounds to freshwater organisms. EPA-600/4-85/014. Springfield, VA:National Technical Information Service, 1985.

34. American Society for Testing and Materials. National guide for conducting the frog embryo teratogenesis assay-Xenopus (Fetal). Report No E 1439–91. Philadelphia:American Society for Testing and Materials, 1991.

35. Weber AM, Hornik RJ, Wemm DJ, Neiheisel TW, Lewis PA, Robidun EL, Medkedick and Kessler F. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Report No 600/4-870/028. Springfield, VA:National Technical Information Service, 1988.

36. Tillitt DE, Kubiak TJJ, Ankley GT, Giesy JP. Dioxin-like toxic potency in Forster’s tern eggs from Green Bay, Lake Michigan, 1988 and 1989. J Great Lakes Res 25:25–38 (1999).

37. Tillitt DE, Ankley GT, Giesy JP, Ludwig JP, Kurita-Matsuba H, Weseloh DV, Ross PS, Bishop CA, Sileo L, Stromborg KL, Larson J, Kubiak TJ. Polychlorinated biphenyl residues and egg mortality in double-crested Cormorants from the Great Lakes. Environ Toxicol Chem 11:1251–1258 (1992).

38. Ankley GT, Tillitt DE, Giesy JP, Jones PD, Verbrugge DA. Bioassay-derived 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalence: an environmental health perspective from the flesh and eggs of Lake Michigan chinook salmon (Oncorhyncus tshawytscha) and possible implications for reproduction. Can J Fish Aquat Sci 48:1685–1690 (1991).

39. Henshel DS, Hemin BM, Vo MT, Cleaves JD. A short-term test for dioxin teratogenicity using chicken embryos. In: Environmental Toxicology and Risk Assessment, ASTM STP 1173 (Gorsuch JW, Dwyer FJ, Ingersoll CG, LaPoint TW, eds). Philadelphia:American Society for Testing and Materials, 1993; 159–174.

40. Walker MK, Hufnagle JR, Clayton MK, Peterson RE. An egg injection method for assessing early life stage mortality of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in rainbow trout. (Oncorhyncus mykiss). Aquat Toxicol 22:15–38 (1992).

41. Walker MK, Spitsbergem JM, Olson JR, Peterson RE. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) toxicity during early life stage development of lake trout (Salvelinus namaycush). Can J Fish Aquat Sci 48:875–883 (1991).

42. Soto AM, Lin T, Justicia H, Silvia R, Sonnenschein C. An “in culture” bioassay to assess the estrogenicity of xenobiotics (E-SCREEN). In: Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection (Colborn T, Clement C, eds). Princeton, NJ:Princeton Scientific Publishing, 1992:295–309.
43. Thomas P, Smith J. Binding of xenobiotics to the estrogenic receptor of spotted seatrout: a screening assay for potential estrogenic effects. Mar Environ Res 35:147-151 (1993).

44. Owens JW, Swanson SM, Birkholz DA. Bioaccumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran and extractable organic chlorine at a bleached-kraft mill site in a northern Canadian river system. Environ Toxicol Chem 13:343-354 (1994).