FOUR

Endocrine Disruptors

STEPHEN SAFE

My initial involvement with endocrine disruptors and the endocrine disruption hypothesis began, unknowingly, in the early 1970s when I was a research officer at the National Research Council of Canada in the Atlantic Regional Laboratory in Halifax, Nova Scotia. Following the identification of polychlorinated biphenyls (PCBs) as highly stable environmental contaminants, my friend and colleague, Otto Hutzinger, persuaded me to collaborate with him on some of the first studies of those chemicals. To investigate concerns that PCBs would not undergo degradation in the environment through chemical/photochemical or metabolic pathways, our initial studies focused on the synthesis of PCB standards that were then used to demonstrate that PCBs undergo photochemical degradation and are metabolized by rats, fish, and

birds.[1] We also carried out similar studies with several different classes of halogenated aromatic pollutants including the infamous and highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) and related compounds.

During the 1980s, my research focused on the structure-activity relationships for TCDD and related compounds, and this led to the development of the toxic equivalency factor approach for risk assessment of "dioxin-like" compounds (including PCBs).[2] Our studies on the mechanism of action of TCDD were greatly influenced by a paper by Kociba and coworkers who reported the results of their two-year ("lifetime") dioxin feeding studies in male and female Sprague-Dawley rats. In this study, male rats did not develop TCDD-induced tumors, whereas in female rats there was a significant increase in liver tumors.[3] Buried within their extensive analysis of each tissue for tumors or precancerous lesions were some intriguing results on uterine and mammary tumors, both of which spontaneously develop in older female rats *that have normal estrogen levels*. Decreases in both tumors were apparent in rats maintained on the TCDD diet, consistent with the idea that TCDD inhibited estrogen-dependent tumor formation and development.

Thus, TCDD exhibited antitumorigenic activity by disrupting

1. S. Safe and O. Hutzinger, "Polychlorinated Biphenyls: Photolysis of 2,4,6,2′,6′ Hexachlorobiphenyl," *Nature* 232 (1971): 641–42; O. Hutzinger et al., "Polychlorinated Biphenyls: Metabolic Behavior of Pure Isomers in Pigeons, Rats, and Brook Trout," *Science* 178 (1972): 312–14; O. Hutzinger et al., "Identification of Metabolic Dechlorination of Highly Chlorinated Biphenyl in Rabbits," *Nature* 252 (1974): 698–99.

2. S. Safe, "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)," *C. R. C. Crit. Rev. Toxicol.* 21 (1990): 51–88.

3. R. J. Kociba et al., "Results of a 2-Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8- Tetrachlorodibenzo-*p*-dioxin (TCDD) in Rats," *Toxicol. Appl. Pharmacol.* 46 (1978): 279–303.

or blocking the formation and growth of age- and estrogen-dependent mammary (and uterine) tumors in rats, and this observation was subsequently confirmed in other laboratory animal studies. Moreover, women accidentally exposed to TCDD in Seveso, Italy, had reduced incidence of breast and endometrial cancers compared to usual rates of these tumors.[4]

TCDD acts through the Ah receptor, a cellular component that binds to the TCDD molecule, and my laboratory has been investigating the unique Ah receptor-mediated antiestrogenic/anticarcinogenic actions of TCDD and developing new nontoxic analogs of TCDD for treating breast cancer.[5] Some of these compounds are in preclinical studies, and we are also investigating their use for treatment of prostate cancer.

My involvement in the endocrine disruptor controversy began in response to an article reporting that PCB and DDE levels were higher in breast cancer patients than in women who did not have the disease, "comparisons" or "controls." It was suggested that the estrogenic activity of organochlorine compounds such as PCBs and DDE may increase the risk for breast cancer.[6] I expressed several concerns regarding this hypothesis, including the fact that the human diet contains contaminants such as TCDD and PCBs, which exhibit antiestrogenic activity, as well as dietary phytochemicals, compounds found in plants that exhibit both es-

4. P. A. Bertazzi et al., "Health Effects of Dioxin Exposure: A 20-year Mortality Study," *Am. J. Epidemiol.* 153 (2001): 1031–44.

5. S. Safe et al., "Selective Ah Receptor Modulators (SAhRMs): Progress Towards Development of a New Class of Inhibitors of Breast Cancer Growth," *J. Women's Cancer* 3 (2001): 37–45; A. McDougal et al., "Tamoxifen-induced Antitumorigenic/Antiestrogenic Action Synergized by a Selective Ah Receptor Modulator," *Cancer Res.* 61 (2001): 3901–7.

6. F. Falck et al., "Pesticides and Polychlorinated Biphenyl Residues in Human Breast Lipids and Their Relation to Breast Cancer," *Arch. Environ. Health* 47 (1992): 143–46.

trogenic and antiestrogenic activity and have been linked to disease prevention.[7]

The Endocrine Disruptor Hypothesis

In the early 1990s, authors of several publications heightened concerns about the potential adverse human health effects associated with background environmental exposures to chemicals that disrupt endocrine signaling pathways.[8] The adverse effects of TCDD and related compounds on wildlife and laboratory animals had already been established,[9] and it was hypothesized that other endocrine-active compounds such as estrogenic chemicals that bind directly to the estrogen receptor (ER) (direct-acting estrogens) may pose environmental and human health problems. Colborn and coworkers also pointed out numerous environmental contaminant-induced wildlife problems, especially those associated with reproduction and development, and suggested that these could be sentinels for ongoing human health problems.[10]

7. S. Safe, "Dietary and Environmental Estrogens and Antiestrogens and Their Possible Role in Human Disease," *Environ. Sci. Pollut. Res.* 1 (1994): 29–33.

8. K. B. Thomas and T. Colborn, "Organochlorine Endocrine Disruptors in Human Tissue," in *Chemically Induced Alterations in Sexual Development: The Wildlife/Human Connection,* T. Colborn and C. Clement., eds. (Princeton, N.J.: Princeton Scientific Publishing, 1992), pp. 365–94; T. Colborn, F. S. Vom Saal, and A. M. Soto, "Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans," *Environ. Health Perspect.* 101 (1993): 378–84; D. J. Hunter and K. T. Kelsey, "Pesticide Residues and Breast Cancer: The Harvest of a Silent Spring," *J. Natl. Cancer Inst.* 85 (1993): 598–99; K. El-Bayoumy, "Environmental Carcinogens That May be Involved in Human Breast Cancer Etiology," *Chem. Res. Toxicol.* 5 (1993): 585–90; R. M. Sharpe and N. F. Skakkebaek, "Are Oestrogens Involved in Falling Sperm Counts and Disorders of the Male Reproductive Tract?" *Lancet* 341 (1993): 1392–95.

9. L. S. Birnbaum, "Developmental Effects of Dioxin," *Environ. Health Perspect.* 103 (1995): 89–94.

10. Colborn et al., "Developmental Effects."

They were particularly concerned about possible effects of *in utero* or early postnatal exposures on the development of the male and female reproductive tracts, which are highly sensitive to steroid hormone levels.

In one of the first studies of data collected about sperm quality over time, Carlsen and coworkers analyzed 61 sperm-count studies from several countries published between 1938 and 1991 and showed that there was "a significant decrease in mean sperm count from 113 $\times$ 10^6/ml in 1940 to 66 $\times$ 10^6/ml in 1990 ($p < 0.0001$)" and concluded that "there has been a genuine decline in semen quality over the past 50 years."[11] Sharpe and Skakkebaek later hypothesized "that the increasing incidence of reproductive abnormalities in the human male may be related to increased oestrogen exposure *in utero*."[12] At about the same time, Mary Wolff and her coworkers[13] reported that either PCBs or 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE, a long-lived degradation product of DDT) levels were elevated in breast cancer patients as compared to levels in controls, and it was subsequently hypothesized that synthetic estrogenic compounds (xenoestrogens) in combination with genetic factors may be preventable causes of breast cancer.[14]

The observed wildlife responses coupled with indications of a worldwide decrease in sperm counts and reports of higher PCB/DDE levels in breast cancer patients (vs. controls) immediately captured the attention not only of scientists and government reg-

11. E. Carlsen et al., "Evidence for the Decreasing Quality of Semen during the Past 50 Years," *Br. Med. J.* 305 (1992): 609–12.

12. Sharpe and Skakkebaek, "Oestrogens."

13. Falck et al., "Pesticides"; M. S. Wolff et al., "Blood Levels of Organochlorine Residues and Risk of Breast Cancer," *J. Natl. Cancer Inst.* 85 (1993): 648–52.

14. D. L. Davis et al., "Medical Hypothesis: Xenoestrogens as Preventable Causes of Breast Cancer," *Environ. Health Perspect.* 101 (1993): 372–77.

ulators but also of the media and public. Numerous reports on television and in newspapers and magazines highlighted decreased sperm counts, smaller penises (in alligators living in a pond near a Superfund site), and chemical-induced breast cancer. Perhaps the classic statement belongs to Dr. Louis Guillette of alligator-penis fame, who informed a congressional panel that "every man in this room is half the man his grandfather was."

In her article entitled "Hormone Hell" in *Discover Magazine* (in September 1996), Catherine Dold wrote: "Industrial chemicals —from plastics to pesticides—paved the road to modern life. Now it appears that these same chemicals, by mimicking natural hormones, can wreak havoc in developing animals. And the road we once thought led to material heaven is heading somewhere else entirely."

Lawrence Wright in his *New Yorker* article (January 15, 1996) entitled "Silent Sperm" extensively discusses the falling sperm count issue and the studies by Skakkebaek, Sharpe, and their colleagues. Mr. Wright also mentions an interview with Dr. Harry Fisch who "claims that his work refutes the whole notion of a decline in the world's sperm count." Unfortunately, Dr. Fisch's paper could not be released prior to its publication and therefore the impact of his work was not fully appreciated.

My comments on the sperm count issue and the role of estrogens were among the few statements in this (and many other) articles that disputed the hypothesis:

> "The estrogen link is total bunk," Stephen Safe, a professor of toxicology at Texas A&M University told me. . . . Safe admitted that he didn't have a clue to what could be causing lower sperm counts and other male reproductive problems. "Lord only knows," he said. "It may be a very regional thing. But just because Denmark has a problem and a few alligators in a swamp below a Superfund site develop small penises doesn't mean our sperm counts are going down or our re-

productive success has declined. I just don't think we should extrapolate."

Other early articles in *Newsweek* (March 21, 1994, "The Estrogen Complex"), *Time* (March 18, 1996, "What's Wrong with Our Sperm?"), *Science News* (January 8, 1994, "The Gender Benders," and January 22, 1994, "That Feminine Touch"), and a British television special entitled "Assault on the Male" forecast a gloomy future for mankind!

Not surprisingly, environmental and health research and regulatory agencies in most developed countries have issued lengthy reports on endocrine disruptors, and review articles on every aspect of this hypothesized problem have appeared in scientific journals. In addition, increased funding for research on endocrine disruptors has resulted in new data as well as the generation of several controversies regarding interpretation of laboratory animal and cell culture data from different laboratories. Those results and controversies continue to attract media attention. In contrast, results from human studies have been less controversial and somewhat reassuring; however, reporting of these data has been minimal. Who cares if we are more than half the men our grandfathers were! Unfortunately, many in the news media fail to report good news on environmental issues, which is a disservice to their readers/viewers.

Endocrine Disruptors and Male Reproductive Capacity

The initial report suggesting a worldwide decrease in sperm counts coupled with a hypothesis that this may be part of larger syndrome (i.e., decreased male reproductive capacity)[15] spurred research efforts around the world. In addition to sperm counts,

15. Sharpe and Skakkebaek, "Oestrogens"; Carlsen et al., "Evidence."

scientists have investigated other potential indicators of diseases/problems associated with the male reproductive tract including testicular cancer, prostate cancer, fertility, male/female birth ratios, hypospadias (displaced urethral opening), and cryptorchidism (undescended testicles) in infants.

All but one of these studies addressed only changes over time and did not attempt to measure exposures to chemicals. The one study that attempted to correlate levels of exposure to endocrine disruptor chemicals with an adverse response examined testicular cancer. As summarized in this section, it should be clear that facts do not support the frightening "assault on the male" scenarios presented in the media and by some scientists.

Sperm Counts

The issue of time-dependent decreases or increases in sperm counts had been frequently raised prior to the report by Carlsen and coworkers on their meta-analysis of 61 selected sperm count studies.[16] Their work was highly provocative, and the results of their meta-analysis study have been hotly debated by academic and nonacademic scientists, and the difficulties in obtaining consistent sperm count/quality data have also been documented.

Since 1993, there has been a host of new studies on sperm counts and quality from men at various clinics (Table 1).[17] Results

16. Carlsen et al., "Evidence."

17. D. A. Adamopoulos et al., "Seminal Volume and Total Sperm Number Trends in Men Attending Subfertility Clinics in the Greater Athens Area During the Period 1977–1993," *Hum. Reprod.* 11 (1996): 1936–41; I. S. Tummon and D. Mortimer, "Decreasing Quality of Semen," *Br. Med. J.* 305 (1992): 1228–29; J. Auger et al., "Decline in Semen Quality Among Fertile Men in Paris During the Past 20 Years," *N. Engl. J. Med.* 332 (1995): 281–85; S. Irvine et al., "Evidence of Deteriorating Semen Quality in the United Kingdom: Birth Cohort Study in 577 Men in Scotland Over 11 Years," *Br. Med. J.* 312 (1996): 467–71; K. Van Waeleghem et al., "Deterioration of Sperm Quality in Young Healthy Belgian Men," *Hum. Reprod.* 11 (1996): 325–29; J. Gyllenborg

Table 1. Sperm Counts/Quality Studies: 1993–Present

Cohort	*Location*	*Years of Data Collection*	*Sperm Counts (10^6/ml)*
A. DECREASED SPERM COUNTS/QUALITY			
Fertility clinic (20)	Greece (Athens)	1977–1993	51–39
Husbands (infertile women) (21)	UK (London)	1978–1989	101–76
Sperm donors (22)	France (Paris)	1973–1992	89–60
Sperm donors (23)	Scotland	birth cohort	98–78
Infertile men (24)	Belgium	birth cohort (1950–1970)	—
B. NO CHANGE OR SLIGHTLY INCREASED SPERM COUNTS/QUALITY			
Volunteer donors (25)	Denmark (Copenhagen)	1977–1995	53–72.7
Infertile couples (26)	Venezuela (Merida)	1981–1995	—
Volunteer donors (27)	Australia (Sydney)	1980–1995	69
Husbands (infertile women) (28)	Denmark (Odense)	birth cohort[a] (1950–1970)	183.7
Husbands (infertile women) (29)	Slovenia	1983–1996	81
Fertility clinics (30)	Spain (Barcelona)		44
Vasectomy clinics (31)	New York	1970–1994	131.5
	California	1970–1994	72.7
	Minnesota	1979–1994	100.8
Sperm donors (32)	France (Toulouse)	1977–1992	68.4
Sperm donors (33)	Washington (State)	1972–1993	52
Sperm donors (34)	Japan (Sapporo)	1975–1998	70.9–79.6
C. VARIABLE RESULTS–DEPENDING ON SELECTION OF TIME PERIOD			
Infertile men (35)	Denmark	1950–1971 (decrease)	
		1922–1971 (no change)	
Fertility clinics (36)	Canada	1984–1996 (decrease)	variable
		1975–1996 (no change)	

Note: a. This approach presents sperm counts based on a defined range of birth dates (e.g., 1950–1970) for individuals in a study.

from some clinics indicated decreased sperm quality; however, most studies indicate that there has not been a significant decline in sperm quality during the last fifteen to twenty-five years.

The work by Fisch and coworkers on sperm quality of men from vasectomy clinics in New York, California, and Minnesota revealed no change in sperm counts, sperm volume, or sperm motility in the period 1970 through 1994.[18] They did, however, show surprisingly large differences in sperm counts between the three locations. Sperm counts in New York, California, and Minnesota were 131.5, 72.7, and 100.8 $\times$ 10^6/ml, respectively, and still

et al., "Secular and Seasonal Changes in Semen Quality Among Young Danish Men: A Statistical Analysis of Semen Samples from 1927 Donor Candidates during 1977–1995," *Int. J. Androl.* 22 (1999): 28–36; I. Tortolero et al., "Semen Analysis in Men from Merida, Venezuela, Over a 15-Year Period," *Arch. Androl.* 42 (1999): 29–34; D. J. Handelsman, "Sperm Output of Healthy Men in Australia: Magnitude of Bias Due to Self-selected Volunteers" *Human Reprod.* 12 (1997): 101–5; P. E. Rasmussen, K. Erb, and L. G. Westergaard, "No Evidence for Decreasing Semen Quality in Four Birth Cohorts of 1,055 Danish Men Born Between 1950 and 1970," *Fertil. Steril.* 68 (1997): 1059–69; B. Zorn et al., "Semen Quality Changes Among 2343 Healthy Slovenian Men Included in an IVF-ET Programme from 1983 to 1996," *Int. J. Androl.* 22 (1999): 178–83; P. Andolz, M. A. Bielsa, and J. Vila, "Evolution of Semen Quality in North-Eastern Spain: A Study in 22,759 Infertile Men Over a 36-Year Period," *Hum. Reprod.* 14 (1999): 731–35; H. Fisch et al., "Semen Analyses in 1,283 Men from the United States Over a 25-Year Period: No Decline in Quality. *Fertil. Steril.* 65 (1996): 1009–14; L. Bujan et al., "Time Series Analysis of Sperm Concentration in Fertile Men in Toulouse, France Between 1977 and 1992," *Br. Med. J.* 312 (1996): 471–72; C. A. Paulsen, N. G. Berman, and C. Wang, "Data from Men in Greater Seattle Area Reveal No Downward Trend in Semen Quality: Further Evidence that Deterioration of Semen Quality Is Not Geographically Uniform," *Fertil. Steril.* 65: (1996): 1015–20; N. Itoh et al., "Have Sperm Counts Deteriorated Over the Past 20 Years in Healthy, Young Japanese Men? Results from the Sapporo Area," *J. Androl.* 22 (2001): 40–44; Y. Zheng et al., "Is Semen Quality Related to the Year of Birth Among Danish Infertility Clients?" *Int. J. Epidemiol.* 26 (1997): 1289–97; E. V. Younglai, J. A. Collins, and W. G. Foster, "Canadian Semen Quality: An Analysis of Sperm Density Among Eleven Academic Fertility Centers," *Fertil. Steril.* 70 (1998): 76–80.

18. Fisch et al., "Semen analyses."

lower sperm counts, 52 $\times$ 10^6/ml, were reported from the state of Washington,[19] indicating large demographic differences in sperm counts within the United States. Such variability has also been observed in other countries.[20] The effects of geographic differences on sperm counts were particularly striking in Canada, where the values from eleven different centers ranged from 51–121 $\times$ 10^6/ml in 1984 and 48–137 $\times$ 10^6/ml in 1996.[21]

These results suggest that persistent organic pollutants (POPs), such as PCBs, DDE, and other organochlorine pesticides, are unlikely to be causative agents for decreases in sperm counts (geographic or temporal) because human levels of these environmental contaminants tend to be similar within most countries except for a few specific groups (e.g., people whose diets include lots of fish). Since sperm counts in males are highly variable and are influenced by many different factors, this parameter may not be a useful indicator for determining potential adverse exposures to environmental endocrine disruptors. Nevertheless, results of more recent studies in Japan indicate that sperm counts are not decreasing in many areas.[22] Future studies that investigate differences in sperm counts within various countries and regions may provide new insights on sperm-count variability.

Fertility

Temporal changes in fertility may be a more reliable indicator than sperm counts regarding possible alterations in male reproductive capacity, and the World Health Organization has devel-

19. Paulsen et al., "Data."
20. Younglai et al., "Canadian Semen Quality"; J. Auger and P. Jouannet, "Evidence for Regional Differences of Semen Quality Among Fertile French Men," *Hum. Reprod.* 12 (1997): 740–45.
21. Younglai et al., "Canadian Semen Quality."
22. Itoh et al., "Sperm Counts."

oped protocols for determining human fertility changes.[23] At least two studies have investigated the effects of *in utero* exposure to pharmacologic (high) doses of estrogen (with or without progestins) or the potent synthetic estrogenic drug diethylstilbestrol (DES) on the fertility of male offspring.

In the 1950s and early 1960s, estrogens and DES were prescribed for women experiencing problems during pregnancy, and a study in Chicago in the early 1960s investigated the effects of DES on pregnancy outcomes by comparing outcomes in women who received DES to outcomes in a control group of women who received a placebo. After data became available that demonstrated harmful effects of DES, Wilcox and coworkers contacted the sons of women in this study to evaluate the long-term effects of DES exposure on their fertility.[24] Based on their analyses, Wilcox and coworkers concluded that "High doses of DES did not lead to impairment of fertility or sexual function in adult men who had been exposed to the drug *in utero*."

Lamuela-Raventos and coworkers studied a group of men and women in Finland (1954–63) exposed *in utero* to pharmacologic doses of estrogens alone or estrogens/progestins (combined) and concluded that these "drugs as used in the study population did not have much impact on the fertility of offspring."[25] These data, coupled with studies showing no decrease in fertility in Sweden and Britain,[26] indicate that there is not a global decrease in male fertility.

23. T. M. Stewart et al., "Feasibility of Surveillance of Changes in Human Fertility and Semen Quality" *Hum. Reprod.* 16 (2001): 177–87.

24. A. J. Wilcox et al., "Fertility in Men Exposed Prenatally to Diethylstilbestrol," *N. Engl. J. Med.* 332 (1995): 1411–16.

25. R. M. Lamuela-Raventos et al., "Direct HPLC Analysis of *Cis*- and *Trans*-resveratrol and Piceid Isomers in Spanish Red *Vitis vinifera* Wines," *J. Agric. Food Chem.* 43 (1995): 281–83.

26. O. Akre et al., "Human Fertility Does Not Decline: Evidence from

Sex Ratios at Birth

Davis and coworkers examined birth sex ratios in several industrial countries and reported that the "usual" 1.06:1.0 male to female ratio had declined.[27] Their conclusions stated, "We propose that reduced male proportion at birth be viewed as a sentinel health event that may be linked to environmental factors," and as a potentially useful measurement for determining the role and identities of endocrine active chemicals that could affect birth sex ratios. Interestingly, some recent studies indicate that the male birth fraction is dependent on multiple factors including race, parental age, and birth weight. A study in Finland investigated sex ratios in that country over a period of 250 years (1751–1997), and concluded that decreased sex ratios have not been observed since 1920.[28] Moreover, after examination of multiple parameters including chemical usage and human levels of organochlorine contaminants, they concluded that "we were not able to confirm that chemicalization (in the sense of exposure to agricultural or industrial chemicals) is a significant source of changes in sex ratio."[29]

Sex ratios were determined in families who were accidentally exposed to high levels of dioxin (in Zone A) as a result of an industrial accident that occurred in Seveso, Italy, in 1976.[30] From

Sweden," *Fertil. Steril.* 71 (1999): 1066–69; M. Joffe, "Time Trends in Biological Fertility in Britain," *Lancet* 355 (2000): 1961–65.

27. D. L. Davis, M. B. Gottlieb, and J. R. Stampnitzky, "Reduced Ratio of Male to Female Births in Several Industrial Countries: A Sentinel Health Indicator," *JAMA* 279 (1998): 1018–23.

28. T. Vartiainen, L. Kartovaara, and J. Tuomisto, "Environmental Chemicals and Changes in Sex Ratio: Analysis Over 250 Years in Finland," *Environ. Health Perspect.* 107 (1999): 813–15.

29. Ibid.

30. P. Mocarelli et al., "Change in Sex Ratio with Exposure to Dioxin," *Lancet* 348 (1996): 409.

April 1977 to December 1984 there was a decrease in the male/female sex ratio (26/48); in contrast, from 1985 to 1994, this ratio increased to normal values (60/64). These results suggest that high-level exposure to TCDD may affect birth sex ratios; however, no changes in sex ratios have been observed as a result of parental occupational exposure to relatively high doses of TCDD,[31] and no other data corroborate the Seveso findings.

Hypospadias and Cryptorchidism

It has been hypothesized that hypospadia and cryptorchidism in newborns may also be contributors to a global decrease in male reproductive capacity. Paulozzi has summarized studies of international trends in the rates for those conditions, which were highly variable among different countries.[32] For example, in 1990, hypospadias for the following countries varied from 38 to 7 per 10,000 births, with the United States > Australia > Sweden > Norway > New Zealand > Netherlands > Finland > Japan, and there were also differences within countries. Inter-country variability was also observed for cryptorchidism. There are, however, no correlations in the rates of the two birth defects in various countries.

Paulozzi indicated that the increases in hypospadias "leveled off in many systems after 1985," whereas for cryptorchidism "since 1985, rates declined in most systems" ("systems" refers to health systems in countries/regions that collect these data). Paulozzi suggested that "it is unlikely that further inspection of inter-

31. T. M. Schnorr et al., "Spontaneous Abortion, Sex Ratio, and Paternal Occupational Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin," *Environ. Health Perspect.* 109 (2001): 1127–32.

32. L. J. Paulozzi, "International Trends in Rates of Hypospadias and Cryptorchidism," *Environ. Health Persp.* 107 (1999): 297–302.

national trends alone will shed additional light on the question of endocrine disruption as a cause of birth defects."

Testicular Cancer

The incidence of testicular cancer has been increasing in most countries, and since the risks are highest among younger men, it is possible that initiation of this tumor could be related to *in utero*/early postnatal exposure to some unknown factors including estrogens.[33] There are large differences in the incidence rates of testicular cancer in various countries, and such variability in rates is a common observation in studies of many male reproductive tract problems. For example, between 1985 and 1989, the incidence rates for testicular cancer in highly susceptible thirty-to-thirty-four-year-olds was 2.7, 3.3, 3.4, 5.6, 5.9, 11.1, 18.2, 22.2, and 24.5 per 10^5 in Lithuania, Latvia, Estonia, Finland, Poland, Sweden, Norway, East Germany, and Denmark, respectively.[34] Differences among these northern European countries was >9-fold, and among the Scandinavian countries, there was a >4-fold difference between Denmark (high) and Finland (low).

Sharpe suggested that DDE (which inhibits male sex hormones—androgens—and is an "antiandrogen") may play a role in the hypothesized increases in diseases or problems in the male reproductive tract.[35] However, breast-milk levels of DDE (a commonly used measure for DDE exposures) are comparable in all four Scandinavian countries and therefore do not correlate with

33. R. H. Depue, M. C. Pike, and B. E. Henderson, "Estrogen Exposure During Gestation and Risk of Testicular Cancer," *J. Natl. Cancer Inst.* 71 (1983): 1151–55.

34. R. Bergstrom et al., "Increase in Testicular Cancer Incidence in Six European Countries: A Birth Cohort Phenomenon," *J. Natl. Cancer Inst.* 88 (1996): 727–33.

35. R. M. Sharpe, "Reproductive Biology. Another DDT Connection," *Nature* 375 (1995): 538–39.

different incidence rates for testicular cancer in these countries.[36] This investigation of a possible linkage of an environmental chemical and a specific disease of the male reproductive tract found no support for an association of DDE with the development of testicular cancer.

Summary

The hypothesis that environmental endocrine disruptors may contribute to diseases of the male reproductive tract has spurred considerable research on this area, with a particular emphasis on changes that have occurred over time. There are no apparent global changes in sperm counts and fertility, rates of hypospadias and cryptorchidism, and birth sex ratios. Testicular cancer is increasing in most countries, but it is not correlated with other indicators of male reproductive capacity. Moreover, testicular cancer is increasing while DDE and other POPs are decreasing, suggesting that exposure to these compounds is not linked to testicular cancer.

For many of these responses, there are large differences in incidence rates between and within various countries, and possible etiologic factors that can account for these differences are unknown. Persistent organic pollutants that bioaccumulate are not highly variable within most countries/regions and therefore cannot be responsible for the observed demographic-dependent differences in incidence rates. Research designed to study the reason for region-specific differences in diseases/problems in the male reproductive tract will require new hypotheses and paradigms that include genetic susceptibility, diet, lifestyle factors, and

36. A. Ekbom, A. Wicklund-Glynn, and H. O. Adami, "DDT and Testicular Cancer," *Nature* 347 (1996): 553–54.

other environmental exposures (including chemical contaminants).

Role of PCBs/DDE in Breast Cancer

The reports of Falck and coworkers[37] and Wolff and coworkers[38] that levels of PCBs or DDE were higher in breast cancer patients compared to controls in two cohorts from Connecticut and New York raised concerns that such persistent xenoestrogens (estrogens that originate outside the body, and are often used to denote synthetic estrogens) may play a role in development of breast cancer. Other authors and I[39] criticized the xenoestrogen-breast cancer hypothesis because PCBs/DDE are not mammary carcinogens in high-dose human exposures or in animal tests and some PCBs exhibit antiestrogenic activity in female rats.[40] Subsequent studies on cohorts of breast cancer patients and controls in several

37. Falck et al., "Pesticides."

38. Wolff et al., "Blood Levels."

39. S. Safe, "Environmental and Dietary Estrogens and Human Health —Is There a Problem?" *Environ. Health Perspect.* 103 (1995): 346–51; U. G. Ahlborg et al., "Organochlorine Compounds in Relation to Breast Cancer, Endometrial Cancer, and Endometriosis: An Assessment of the Biological and Epidemiological Evidence," *Crit. Rev. Toxicol.* 25 (1995): 463–531.

40. Ahlborg et al., "Organochlorine Compounds"; K. C. Silinskas and A. B. Okey, "Protection by 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane(DDT) Against Mammary Tumors and Leukemia During Prolonged Feeding of 7,12-dimethylbenz(a)anthracene to Female Rats," *J. Natl. Cancer Inst.* 55 (1975): 653–57; J. D. Scribner and N. K. Mottet, "DDT Acceleration of Mammary Gland Tumors Induced in the Male Sprague-Dawley Rat by 2-acetamidophenanthrene," *Carcinogenesis*2 (1981): 235–39; S. Safe, "Modulation of Gene Expression and Endocrine Response Pathways by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and Related Compounds," *Pharmacol. Therap.* 67 (1995): 247–81; T. Zacharewski, and S. Safe, "Antiestrogenic Activity of TCDD and Related Compounds," in K. S. Korach, ed., *Reproductive and Developmental Toxicology* (New York: Marcel Dekker, 1998), pp. 431–48.

countries have demonstrated that total PCBs and DDE levels were not elevated in patient groups.[41]

41. N. Krieger et al., "Breast Cancer and Serum Organochlorines: A Prospective Study Among White, Black, and Asian Women" *J. Natl. Cancer Inst.* 86 (1994): 589–99; L. López-Carrillo et al., "Dichlorodiphenyltrichloroethane Serum Levels and Breast Cancer Risk: A Case-control Study from Mexico," *Cancer Res.* 57 (1997): 3728–32; P. Van't Veer et al., "DDT (Dicophane) and Postmenopausal Breast Cancer in Europe: Case Control Study," *Br. Med. J.* 315 (1997): 81–85; D. J. Hunter et al., "Plasma Organochlorine Levels and the Risk of Breast Cancer," *New Engl. J. Med.* 337 (1997): 1253–58; A. Schecter et al., "Blood Levels of DDT and Breast Cancer Risk Among Women Living in the North of Vietnam," *Arch. Environ. Contam. Toxicol.* 33 (1997): 453–56; K. B. Moysich et al., "Environmental Organochlorine Exposure and Postmenopausal Breast Cancer Risk," *Cancer Epidemiol. Biomarkers. Prev.* 7 (1998): 181–88; A. P. Hoyer et al., "Organochlorine Exposure and Risk of Breast Cancer," *Lancet* 352 (1998): 1816–20; S. Guttes et al., "Chlororganic Pesticides and Polychlorinated Biphenyls in Breast Tissue of Women with Benign and Malignant Breast Disease," *Arch. Environ. Contam. Toxicol.* 35 (1998): 140–47; G. Liljegren et al., "Case-control Study on Breast Cancer and Adipose Tissue Concentrations of Congener Specific Polychlorinated Biphenyls, DDE and Hexachlorobenzene," *Eur. J. Cancer Prev.* 7 (1998): 135–40; K. J. Helzlsouer et al., "Serum Concentrations of Organochlorine Compounds and the Subsequent Development of Breast Cancer," *Cancer Epidemiol. Biomarkers. Prev.* 8 (1999): 525–32; J. F. Dorgan, "Serum Organochlorine Pesticides and PCBs and Breast Cancer Risk: Results from a Prospective Analysis," *Cancer Causes and Control* 10 (1999): 1–11; G. A. S. Mendonca et al., "Organochlorines and Breast Cancer: a Case-control Study in Brazil," *Int. J. Cancer* 83 (1999): 596–600; E. M. Ward et al., "Serum Organochlorine Levels and Breast Cancer: A Nested Case-control Study of Norwegian Women," *Cancer Epidemiol. Biomarkers. Prev.* 9 (2000): 1357–67; D. Bagga et al., "Organochlorine Pesticide Content of Breast Adipose Tissue from Women with Breast Cancer and Control Subjects," *J. Natl. Cancer Inst.* 92 (2000): 750–53; I. Romieu et al., "Breast Cancer, Lactation History, and Serum Organochlorines," *Am. J. Epidemiol.* 152 (2000): 363–70; R. Millikan et al., "Dichlorodiphenyldichloroethene, Polychlorinated Biphenyls, and Breast Cancer Among African-American and White Women in North Carolina," *Cancer Epidemiol. Biomarkers. Prev.* 9 (2000): 1233–40; S. D. Stellman et al., "Breast Cancer Risk in Relation to Adipose Concentrations of Organochlorine Pesticides and Polychlorinated Biphenyls in Long Island, New York," *Cancer Epidemiol. Biomarkers. Prev.* 9 (2000): 1241–49; T. Zheng et al., "Risk of Female Breast Cancer Associated with Serum Polychlorinated Biphenyls and 1,1-dichloro-2,2'-bis(*p*-chlorophenyl)ethylene," *Cancer Epidemiol. Bio-*

Some investigators have used high-resolution analytical techniques to show that one or more individual PCB congeners or other organochlorine pesticides (e.g., dieldrin) were elevated in breast cancer patients, but these increases have not been observed in other studies. For example, Hoyer and coworkers reported that dieldrin levels were higher in a cohort of Danish breast cancer patients and were inversely correlated with breast cancer survival.[42] In contrast, serum levels of dieldrin were not elevated in Norwegian breast cancer patients[43] or in patients from Missouri.[44] Similar inconsistencies between studies have been observed for PCBs where PCB congeners (but not mixtures) were higher in patients vs. controls.

Studies from several countries have vindicated early skepticism about the postulated causal role of PCBs and DDE in the development of breast cancer. Dr. Mary Wolff, a coauthor of the two initial studies showing higher levels of PCBs and/or DDE in breast cancer patients, was also involved in several of the later

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markers. Prev. 9 (2000): 167–74; T. Zheng et al., "Breast Cancer Risk Associated with Congeners of Polychlorinated Biphenyls," *Amer. J. Epidemiol.* 152 (2000): 50–58; A. P. Hoyer et al., "Organochlorine Exposure and Breast Cancer Survival," *J. Clin. Epidemiol.* 53 (2000): 323–30; T. R. Holford et al., "Joint Effects of Nine Polychlorinated Biphenyl (PCB) Congeners on Breast Cancer Risk," *Int. J. Epidemiol.* 29 (2000): 975–82; M. S. Wolff et al., "Organochlorine Exposures and Breast Cancer Risk in New York City Women," *Environ. Res.* 84 (2000): 151–61; F. Laden et al., ",1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene and Polychlorinated Biphenyls and Breast Cancer: Combined Analysis of Five U.S. Studies," *J. Natl. Cancer Inst.* 93 (2001): 768–76; F. Laden et al., "Plasma Organochlorine Levels and the Risk of Breast Cancer: An Extended Follow-up in the Nurses' Health Study," *Int. J. Cancer* 91 (2001): 568–74; K. J. Aronson et al., "Breast Adipose Tissue Concentrations of Polychlorinated Biphenyls and Other Organochlorines and Breast Cancer Risk," *Cancer Epidemiol. Biomarkers. Prev.* 9 (2000): 55–63.

42. Hoyer et al., "Organochlorine Exposure and Risk"; Hoyer et al., "Oganochlorine Exposure and Survival."

43. Ward et al., "Serum Organochlorine."

44. Dorgan et al., "Serum Organochlorine."

studies and one of these reports concluded, "combined evidence does not support an association of breast cancer risk with plasma/serum concentrations of PCBs or DDE."[45]

Endocrine Disruptors—Personal Reminiscences

My participation in the debate on environmental endocrine disruptors and their potential adverse impacts on human health has been a learning experience. During the 1970s and early 1980s, my research on PCBs and related compounds and the TEF concept contributed to the development of regulatory measures that have resulted in reduced emissions and environmental levels of these compounds. This research was primarily supported by federal funding agencies (the Environmental Protection Agency and the National Institute of Environmental Health Sciences).

Although I am still concerned about environmental impacts of organochlorine pollutants and some endocrine disruptors, I have remained skeptical of the hypothesis that these chemicals are currently having global impact on human health. My skepticism is reinforced by the recently published scientific data that have been referenced in this chapter. My views are also due, in part, to the concepts put forward by Bruce Ames and Lois Gold, who pointed out that the human diet contains multiple toxins and carcinogens that occur naturally in food or are formed during cooking.[46] Moreover, levels and often the potencies of "natural" carcinogens in the diet are far higher than those of carcinogenic industrial contaminants. A similar argument also holds true for endocrine disruptors where dietary intakes of phytoestrogens,

45. Laden et al., "1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene."

46. B. N. Ames and L. S. Gold, "Environmental Pollution, Pesticides, and the Prevention of Cancer: Misconceptions," *FASEB J.* 11 (1997): 1041–52, and see also Ames and Gold chapter, this volume.

and other endocrine-active substances including Ah receptor-active compounds, far outweigh the intakes of endocrine-active manmade environmental contaminants.

Unlike many other scientific controversies, the endocrine disruptor issue has engendered partisan and inflammatory debate on both sides of the issue. My views and statements contributed to this problem, particularly in two articles written as editorials in the *Wall Street Journal* (August 20, 1997) and the *New England Journal of Medicine*.[47] Both articles commented on recently published data that clearly did not support the endocrine disruptor hypothesis, and it was (and is) my view that scientists and the public should be made aware of these results and their significance.

I drew attention to the extensive worldwide coverage in 1996–97 of a report in *Science* indicating that combinations of weakly active estrogenic pesticides interacted synergistically and that this observation strongly supported the endocrine disruptor hypothesis. Scientists in my laboratory, among many others, had not observed these interactions, and about a year later, the authors of the *Science* paper withdrew it, stating that they had been unable to reproduce their own results. In contrast to zealously reporting the original finding, the media paid scant attention to scientific data showing "no synergism," and I believed (and believe) that it was important to point this out.

The *Wall Street Journal* article resulted in a less than complimentary letter from a member of the National Research Council (NRC) panel on endocrine disruptors to NRC staff indicating that "Safe has undermined the work of the panel" and has "contaminated the pending report." Needless to say, there were demands for my removal from the panel, and the letter asserted that my

47. S. Safe, "Xenoestrogens and Breast Cancer," *N. Engl. J. Med.* 337 (1997): 1303–4.

article was part of a conspiracy linked to "specific interest groups that Safe represents."

At that time (1994–96), I had research support for a project on estrogenic compounds funded by the Chemical Manufacturers Association (CMA); my only official contact with the association was Ann Mason, Director of Scientific and Regulatory Affairs (Chlorine Chemistry Council, CMA), who asked for a yearly report. My opinions on the endocrine disruptor hypothesis have been based on analysis of scientific publications and have been consistent prior to, during, and after the research (not personal) support from the CMA.

The editorial in the *New England Journal of Medicine* (*NEJM*) commented on an article that showed that plasma DDE levels in breast cancer patients from several states in this country were not significantly different from levels in control patients.[48] Similar results have been reported in other studies,[49] and my final comments pointed out that "it is incumbent on scientists, the media, legislators, and regulators to distinguish between scientific evidence and hypothesis, and not to allow a 'paparazzi science' approach to these problems." The editors received several negative reactions to my article and these included complaints that I had not disclosed my financial interests.

At the time, based on the then-current *NEJM* guidelines, which asked for current support, I had not declared my previous grant support from the CMA. In retrospect, I agree that full disclosure, even of potential conflicts, is the best course and I should have been more perceptive of this issue.

The subject of endocrine disruptors and fear of chemicals (chemophobia) has been addressed in several recent books on both sides of this contentious issue. *Our Stolen Future; Hormone*

48. Hunter et al., "Plasma Organochlorine."
49. See note 41.

Deception; Hormonal Chaos; and *The Feminization of Nature—Our Future at Risk* chronicle the perceived, predicted, and observed problems associated with endocrine disruptors.[50] Skepticism regarding the human impact of environmental contaminants has been discussed in several books including *The Skeptical Environmentalist* and *Naturally Dangerous: Surprising Facts About Food, Health and the Environment*,[51] and John Stossel (ABC television) remains a consistent skeptic with his features on junk science.

The concern regarding human exposure to relatively low environmental levels of estrogenic contaminants and other endocrine disruptors must take into account higher exposures to phytoestrogens and other naturally occurring endocrine-active compounds in the diet. Although there is evidence linking some wildlife problems to chemical exposures (e.g., organochlorines) that act through endocrine pathways, there have also been surprising observations. Studies in Britain initially raised concern regarding feminization of fish in British rivers, and this was initially linked to estrogenic alkylphenols (industrial products) that contribute to this response in the vicinity of sewage outflows. However, the problems in many of the British rivers where feminization of male fish was observed were not associated with synthetic alkylphenols. Instead, the problem has now been linked

50. See T. Colborn, D. Dumanoski, and J. P. Myers, *Our Stolen Future: Are We Threatening Our Fertility, Intelligence and Survival? A Scientific Detective Story* (New York: Penguin Books, 1996); L. D. Berkson, *Hormone Deception* (Chicago: Contemporary, 2000); S. Krimsky, *Hormonal Chaos: The Scientific and Social Origins of the Environmental Endocrine Hypothesis* (Baltimore: John Hopkins University Press, 2000); and D. Cadbury, *The Feminization of Nature: Our Future at Risk* (London: Penguin Books, 1998).

51. Glassner, B. *The Culture of Fear* (New York: Basic Books, 1999); National Research Council: Committee on Hormonally Active Agents in the Environment, *Hormonally Active Agents in the Environment* (London: Penguin Books, 1999).

to natural estrogens (17β-estradiol/estrone) from human and animal waste and possibly ethynylestradiol, used in birth control pills.[52]

My skeptical comments on the endocrine disruptor hypothesis have been extensively criticized from both a scientific and personal point of view. In the book *Hormonal Chaos*, Dr. Krimsky states: "Safe's role in disputing different components of the hypothesis has also raised eyebrows among some of his colleagues who consider his industrial funding sources a matter of dishonor in these sensitive areas of science." Tony Tweedale (whom I have never met), writing for an environmental group, referred to me as "one loud and inane mouth" and "He'll relatively soon get his come-uppance on these ridiculous arguments of his. . . . I only hope we ensure he gets it good and hard."

Mindless personal attacks by individuals whom you do not know are disappointing, particularly in light of results of continuing studies that have not identified linkages between exposure to endocrine disruptors and human disease. I have always acknowledged the adverse impact of environmental endocrine-active compounds on fish and wildlife populations in some areas, but have questioned their impact on human health. Scientific studies published in the past six to eight years have addressed many of the critical issues associated with endocrine disruptors and human health, and extensive references to these papers have been intentionally included in this chapter. Results of the more recent studies indicate that initial concerns regarding hypothesized endocrine disruptor-induced human problems may not be justified.

52. E. J. Routledge et al., "Identification of Estrogenic Chemicals in STW Effluent. 2. *In vivo* Responses in Trout and Roach," *Environ. Sci. Technol.* 32 (1998): 1559–65; C. Desbrow et al., "Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical Fractionation and In Vitro Biological Screening," *Environ. Sci. Technol.* 32 (1998): 1549–58.

Gregg Easterbrook in an editorial entitled "Science Fiction" in the *New Republic* (August 30, 1999) critically examines the endocrine disruptor issue and concedes that there may be "dangerous endocrine disruptors." However, he concludes his editorial with a statement that is highly relevant: "It's strange to think how quickly speculative, lightly researched claims, advanced by advocates with a fund-raising interest can go straight to the top of the national policy agenda, while so many undeniably genuine problems languish." I do not entirely agree that the endocrine disruptor hypothesis was lightly researched or did not deserve serious scientific study and evaluation by regulatory agencies. The concern with this issue and others is that scientists/regulators develop vested interests in specific problems, and there is great reluctance on their (our) part to say "enough is enough." With limited funding available, this can seriously impede research that addresses more pressing environmental and human health issues.