Environmental Health Sciences
609 East High Street Charlottesville, Virginia 22932

Testimony before the U.S. Senate Committee on Commerce, Science, and Transportation. 14 May 2008.

Base health standards on 21st Century medical science, not 16th Century dogma.

J.P. Myers, Ph.D.
Chief Scientist
Environmental Health Sciences

Large scientific literatures of peer-reviewed publications now plausibly link bisphenol A (BPA) and several phthalates to an array of adverse health outcomes.

For bisphenol A these include prostate and breast cancer, loss of fertility (including via polycystic ovaries and uterine fibroids, as well as reduced sperm count and spontaneous miscarriage) and impaired neurological development. Numerous studies show that many of these effects can be caused in laboratory animals at levels beneath the average concentration found in American serum today.[1]

For phthalates these include abnormalities in the male reproductive tract (including undescended testes, hypospadias and reduced sperm count) as well as heightened sensitivity and reactivity of the immune system, which may lead to hyperallergic reactions and asthma.

The strength of the evidence varies for each of these potential effects, for both phthalates and BPA. The human data on phthalates are stronger; indeed for BPA there are almost no epidemiological studies. But the evidence from animal experiments on BPA, especially at very low doses within the range of common human exposure, is much more extensive than with phthalates. And the mechanism of action of BPA in humans is the same as the mechanism of action in animals. Hence the animal findings are highly relevant to predicting human health impacts.

Despite this evidence, both BPA and phthalates are in widespread, indeed ubiquitous use in commerce today. Virtually all Americans carry measurable levels in their fluids and tissues. None of the relevant federal agencies have taken action to reduce exposures.

Why?

The scientific basis of regulatory toxicology, as it is applied today by federal regulators, rests upon an assumption derived from 16th Century dogma. That assumption, never tested in standard procedures to establish acceptable exposure limits, conflicts directly with 21st Century medical science.

The assumption is that experiments with high doses will reveal the effects of low doses.

It is based upon the 16th Century observation by Paracelsus that "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy." [2] This has been paraphrased to become "the dose makes the poison."

The assumption is directly contradicted by decades of research in the medical science of endocrinology showing that hormonally-active compounds have complicated dose-response curves in which low dose exposures can cause effects unpredictable from high dose experiments. BPA and phthalates are both hormonally-active compounds, called endocrine disrupters (EDCs), and peer-reviewed research has reported these complicated dose-response curves for both substances. Nevertheless, the FDA and EPA continue to depend upon this flawed assumption, which has been repeatedly invalidated in careful scientific studies, in these agencies' development of public health standards for, and regulation of, exposures to EDCs. This misled policy is disastrous, as it will lead to many lost opportunities for improving public health that will have implications for decades, as recent research shows long-term detrimental effects not only on exposed individuals, but even subsequent generations.

Biomonitoring studies conducted by the CDC and others document that wherever samples have been analyzed, people are contaminated with many industrial chemicals, including BPA and phthalates. Of particular concern are the numbers and concentrations of chemicals found in human amniotic fluid, fetal blood, and breast milk, rendering it impossible for a child to be born or to be breast-fed without developmental exposure.

Many of these chemicals are known to interfere with the action of hormones in experimental systems, hormones that are essential for healthy development. With a mandate from Congress, for the last decade the US EPA has been designing regulatory tools to screen and test for contaminants with endocrine effects.[3] To date, this process has failed to fully integrate basic endocrinological principles in its decision-making and instead is relying upon toxicological methods that are inappropriate for EDCs.[4] This led to a significant blind-spot in regulatory standard setting.

Chemical monitoring by the CDC, carefully structured to obtain statistically representative estimates of Americans' exposures, typically reveals median serum or urine concentrations well below those produced by dosing regimens in animal experiments used for regulatory toxicology. Those regimens use high doses under the assumption that the effects of high doses can be used to predict low dose impacts. In fact, the estimates of safe daily human exposure doses for chemicals derived from these procedures are never directly tested, even in laboratory animals. Yet increasingly, epidemiological analyses of biomonitoring data showing associations, sometimes striking, between the low concentrations of chemicals measured in the general public and adverse health conditions. Examples include phthalates and sperm defects[5], reproductive tract abnormalities[6], and obesity[7]; pesticides and sperm count[8]; perchlorate[9] or PCBs[10,11,12] and thyroid function; and persistent organic pollutants and type 2 diabetes[13] and insulin resistance.[14]

These associations should not arise if the safety levels established by high-dose testing

are accurate. Several factors could be contributing to this apparent discrepancy between prediction and observation. One is that epidemiological associations do not reflect causality. A second is that the estimate for safety has been based upon an insensitive endpoint. A third is the potential for additive or synergistic effects of mixtures. I will focus here on a fourth, because it challenges the core assumption of regulation toxicology, that high-dose testing is sufficient to predict low-dose effects. A huge experimental literature amassed over decades of mechanistic research in endocrinology demonstrates that this assumption is fundamentally flawed and is highly vulnerable to missing important low-dose adverse effects.

Paracelsus's observation, above, reflects an intuitively logical concept that the higher the exposure, the greater the impact. Testing with high doses, in this view, should reveal any hazards and do so more efficiently than testing with low doses, because the effects will be stronger and easier to detect. This centuries-old paradigm remains the central tenet of modern regulatory toxicological approaches to studying the health effects of chemicals.

Paracelsus' logic holds if and only if chemicals' effects faithfully follow a monotonic dose-response curve. When toxicologists began to focus on potential health effects of chemicals classified as endocrine disruptors, endocrinologists began to raise questions about the appropriateness of assuming monotonicity in toxicological studies of hormonally-active chemicals used in common household products.

Monotonic vs. non-monotonic dose-response curves.
Non-monotonic curves are often described as 'U shaped' or 'inverted-U' shaped.' Monotonic and non-monotonic refer to changes in the slope of the curve describing dose and response. Monotonic curves may be linear or non-linear, but the slope never reverses from positive to negative or vice-versa. Non-monotonic curves change sign, from positive to negative or vice-versa.

The basis for this concern is that non-monotonicity is a general characteristic of hormones. This issue is so central to hormone action that it is a critical component of determining the dose required for hormonally active drugs; an example is Lupron used to treat reproductive disorders in women and prostate cancer in men, since low doses stimulate while high doses inhibit tumor growth.

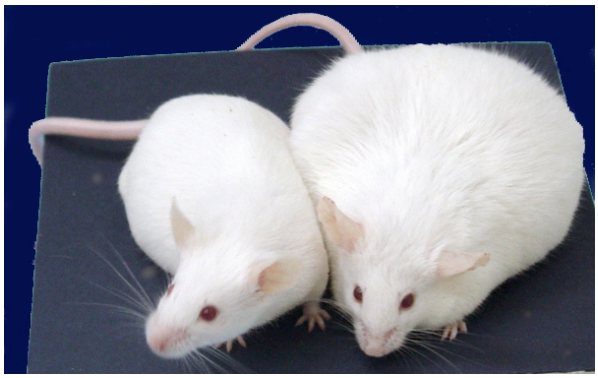
These non-monotonic curves can result from multiple mechanisms, which have been studied by endocrinologists, pharmacologists and neurobiologists for decades. Hormones and hormone-mimicking chemicals act through receptors in target cells. Very low doses can stimulate the production of more receptors (called receptor up-regulation), resulting in an increase in responses, while higher doses (within the typical toxicological range of testing) can inhibit receptors (called receptor down-regulation), resulting in a decrease in responses. The consequence for gene activity, which is regulated by hormone-mimicking chemicals binding to receptors, is that very low doses of these chemicals (in the case of a positively-regulated gene) can up-regulate gene expression, while at higher doses the same chemicals down-regulate gene expression.[1,15] In addition, myriad hormonal feedback mechanisms between the brain, pituitary gland and hormone producing organs (thyroid gland, adrenal glands, ovaries, testes) contribute to the presence of non-monotonic dose-response curves. Equally important, at high doses,

hormones and hormone-mimicking chemicals can bind to receptors for other hormones (e.g., estrogens can interact with androgen and thyroid receptors), producing entirely different effects from those seen at low doses where only binding to estrogen receptors occurs. Also, there is non-specific (non-receptor mediated) toxicity that can occur at high but not low doses. The consequence is that there are qualitative as well as quantitative differences in the effects of high and very low doses of endocrine disrupting chemicals.

Notably, EDCs may also act by mechanisms that do not require direct mediation of classical hormone receptors. For example, they also exert actions upon synthesis or function of enzymes that may be responsible for the synthesis or degradation of hormones; on factors that interact or regulate receptors such as coregulatory factors; and in the case of neurological actions, through neurotransmitter receptors.[16] This concept is important because each of these mechanisms may have a unique dose-response sensitivity to an EDC, adding to the complexity of the overall shape of the dose-response curve.

A recently published example of a non-monotonic response in an animal model, with high biomedical relevance to humans, involves the estrogenic drug diethylstilbestrol (DES), once widely used to treat difficult pregnancies but removed from the market in 1971 because it was found to cause a rare cancer in young adult women who had received fetal exposure. Research has established the BPA is structurally and functionally very similar to DES.

Mice exposed perinatally to relatively high doses of DES (1000 µg/kg/day) had reduced body weight in adulthood, but a much lower dose (1 µg/kg/day) caused adult obesity (figure to right).[17,18]

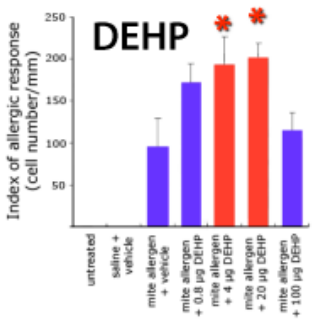


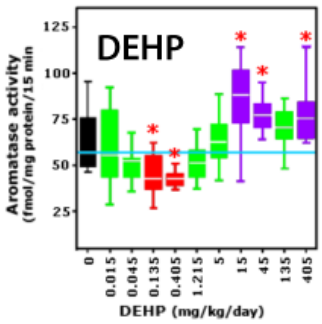
The mouse on the right received the extremely low dose compared to the control on the left. The researchers reported no difference between control and experimental animals in either calories consumed or energy expended.

A similar non-monotonic response has been observed for DES effects on the developing prostate in mice.[19,20,21] A traditional high-dose testing regimen with DES would never have revealed these low-dose effects.

Just as with DES, industrial chemicals that interfere with hormone signaling cannot be expected to follow monotonic dose-response rules. Non-monotonicity has been reported repeatedly for adverse effects with a number of endocrine disrupting compounds, including the bisphenol A, the phthalate DEHP, the pesticides, dieldrin, endosulfan and hexachlorobenzene, the pesticide metabolite DDE, and arochlor 1242, a PCB mixture.[22]

Effects include strong exacerbation of allergic reactions following exposure to DEHP at a concentration one-thousand-fold beneath the current safety standard, which is based on high dose liver toxicity (figure to right),[23] and increased allergic responses caused by picomolar level exposures (parts per trillion) to several persistent organic pollutants.[24] Cells exposed to concentrations of these pollutants a million times higher than the level producing the maximum response showed no effect.





An experiment (figure to left) with rats that involved administration of DEHP was explicitly designed to test the adequacy of high-dose testing.[25] It found that a high dose increased estrogen synthesizing (aromatase) enzyme activity in the brains of neonatal male rats; a dose 100-fold lower appeared to be the "no effect dose", which is used to estimate the dose deemed safe for human exposure (this enzyme is involved in determining sex differences in brain function).

In the experiment above, only because the scientists broke with tradition and also tested lower doses did they find significant down-regulation of aromatase at a dose 37-times lower than the putative no effect dose, an effect opposite to and unpredicted from only testing very high doses.

Other experiments have documented non-monotonicity in rat pituitary cells exposed to pico- through micro-molar levels (parts per trillion to parts per billion) of BPA.[26,27] Acting through a relatively recently discovered estrogen receptor on the surface of the cell membrane, very low picomolar concentrations of the contaminant increased calcium influx and activation of enzyme cascades that dramatically amplify a very low-dose signal into a large cellular response. The dose-response curve followed a strongly non-monotonic, 'inverted-U' shape, with the strongest response at low nanomolar levels. The bioactive concentrations of bisphenol A in these experiments were actually far below the range found ubiquitously in human blood and urine. Another endpoint that follows a non-monotonic pattern is human prostate cancer cell proliferation in response to bisphenol A[28], with the peak response occurring exactly within the range of exposure of men to bisphenol A based on biomonitoring studies.[1,29]

Research over the past 20 years has identified large numbers of endocrine disrupting contaminants that are capable of mimicking or disrupting hormone function. Biomonitoring studies have established that many are widespread contaminants in people. Yet regulatory toxicology as it has been practiced for decades, and as it has been used to set public health exposure standards, ignores non-monotonicity despite the fact that, similar to hormones, all should be expected to display non-monotonic dose-response patterns.

To date the Congressionally-mandated effort by the EPA, called the Endocrine Disruptor Screening Program (EDSP), has not acknowledged these common, indeed standard patterns from endocrinology, and hence it is on course to select methodologies that will remain blind to hazards posed by low doses that lead to adverse effects that only direct low-dose testing can detect.

An effective EDSP is required to protect Americans from exposure to industrial chemicals that can disrupt the endocrine system, which must function properly for normal development to occur as well as for normal adult function. Significant exposure to these chemicals is through the food supply, which is the domain of the FDA, but exposure also occurs through drinking water and air, the domain of the EPA. The American public depends upon these regulatory agencies to set public health standards sufficient to avoid harmful exposures. But until the FDA and EPA move beyond outdated concepts, the public health standards that emerge from their regulatory deliberations will continue to produce a disconnect between what human biomonitoring, epidemiological and mechanistic endocrine studies in animals reveal and what their regulatory decision makers allow.

Were the health implications of these decisions inconsequential, this clash between toxicology and endocrinology would appropriately remain buried in academia. But the range of health conditions now plausibly linked to endocrine-disrupting contaminants— including prostate cancer, breast cancer, attention deficit hyperactivity disorder, infertility (including both male and female reproductive problems), miscarriage, and most recently, hyper-allergic diseases, obesity and type 2 diabetes— makes it imperative that the clash between basic endocrinologists and regulatory toxicologists becomes public and addressed by regulatory agencies. These diseases are major contributors to American's steadily increasing disease burden and to the escalating cost of health care. Extensive, careful and replicable animal research suggests that numerous industrial chemicals to which people are exposed every day, but which have not been adequately studied for health effects in humans, may be significant contributors to these adverse health trends.

As endocrine and reproductive systems are highly conserved between animals and humans, there is no doubt that basic research results on EDCs are directly applicable to human health. Modernizing relevant health standards by incorporating endocrinological principles could help reduce a significant portion of the human disease burden, but this will require regulatory decision makers to begin asking scientifically appropriate questions. The soaring health care crisis in the US demands that the regulatory apparatus of federal government get this right. Blind obedience to 16th century dogma will not solve the problem.

REFERENCES

[1] L.N. Vandenberg, R. Hauser, M. Marcus, N. Olea, W.V. Welshons. Repro. Tox. **24**, 139-177 (2007).
[2] M.A. Gallo, History and Scope of Toxicology, in C.D. Klaassen , *Casarett & Doull's Toxicology, 5th Ed.* (McGraw-Hill, New York, NY, 1996), p. 4.
[3] The 1996 Food Quality Protection Act mandated establishment of the Endocrine Disruptor Screening Program. http://www.epa.gov/scipoly/oscpendo/
[4] A. Gore. Experimental Biol. and Medicine 233, 3 (2008)
[5] R. Hauser, J.D. Meeker, S. Duty, M.J. Silva, A.M. Calafat. Epidemiology **17**, 682-691 (2006).
[6] S Swan *et al*. Environ. Health Perspect. **113**, 1056-1061 (2005).
[7] R .W. Stahlhut, E. van Wijngaarden, T.D. Dye, S. Cook, S.H. Swan. Environ. Health Perspect. **115**, 876-882 (2007).
[8] S. H. Swan et al. Environ. Health Perspect. 111, 1478-1484 (2003).
[9] B.C. Blount, J.L. Pirkle, J.D. Osterioh, L Valentin-Blasini, K.L. Caldwell. Environ. Health Perspect. 114: 1865-1871.
[10] M. E. Turyk, H. A. Anderson, V. W. Persky. Environ. Health. Perspect. **115**, 1197 (2007).
[11] T. Otake *et al.*, Environ. Res. **105**, 240 (Oct, 2007).
[12] J. D. Meeker, L. Altshul, R. Hauser. Environ. Res. **104**, 296 (Jun, 2007).
[13] D-H. Lee, I-K. Lee, K. Song, M. Steffes, W. Toscano, B.A. Baker, D.R. Jacobs. Diabetes Care **29**, 1638-1644 (2006).
[14] D-H. Lee, I-K. Lee, S-H. Jin, M Steffes, D.R. Jacobs, Jr. Diabetes Care **30**, 662-628 (2007).
[15] K.L. Medlock, C.R. Lyttle, N. Kelepouris, E.D. Newman, D.M. Sheehan. 1991. Proc. Soc. Exp. Biol. Med. **196**, 293-300 (1991).
[16] A. C. Gore. Introduction to endocrine-disrupting chemicals, in A.C. Gore, Endocrine-disrupting chemicals: From basic research to clinical practice (Humana Press, New Jersey), pp. 3-8 (2007).
[17] R.R. Newbold, E. Padilla-Banks, R.J. Snyder, WN Jefferson. Birth Defects Research (Part A) **73**, 478-480 (2005).
[18] R.R. Newbold, W. Padilla-Banks, R.J. Snyder, W.N. Jefferson. Mol. Nutr. Food Res. **51**, 912-917 (2007).
[19] F.S. vom Saal, B.G. TImms, M.M. Motano, P. Palanza, K.A. Thayer, S.C. Nagel et al. Proc. Natl. Acad. Sci. USA. **94**, 2056-2061 (1997).
[20] C. Gupta. Proc. Soc. Exp. Biol. Med. **244**, 61-68 (2000).
[21] B.G. Timms. K.L. Howdeshell, L. Barton, S. Bradley, C.A. Richter, F.S. vom Saal. Proc. Natl. Acad. Sci. **102**, 7014-7019 (2005).
[22] J.P. Myers, W. Hessler, EnvironmentalHealthNews.org, 30 April 2007, http://www.environmentalhealthnews.org/sciencebackground/2007/2007-0415nmdrc.html
[23] H. Takano, R. Yanagisawa, K-I. Inoue, T. Ichinose, K. Sadakano, T. Yoshikawa. Environ. Health Perspect. **114**, 1266-1269 (2006).
[24] S. Narita, R.M. Goldblum, C.S. Watson, E.G. Brooks, D.M. Estes, E.M. Curran, T. Midoro-Horiuti. Environ. Health Perspect. 115, 48-52 (2007).
[25] A.J.M. Andrade, S.W. Grande, C.E. Talsness, K. Grote, I. Chahoud. Toxicology **227**, 185-192 (2006).
[26] A.L. Wosniak, N.N. Bulayeva, C.S. Watson. Environ. Health Perspect. **113**, 431-439 (2005).
[27] A. Zsarnofsky, H.H. Lee, H.S. Wang, S.M. Belcher. Endocrinology **146**, 5388-5396 (2005).
[28] Y.B. Wetherill, C.E. Petra, K.R. Monk, A. Puga, K.E. Knudsen, Molec. Cancer Therapeut. **7**, 515-24 (2002)
[29] A.M. Calafat, X. Ye, L-Y. Wong, J.A. Reidy, L.L. Needham. Environ. Health Perspect., in press, doi:10.1289/ehp.10605 (2008).