Environmentally Relevant Xenoestrogen Tissue Concentrations Correlated to Biological Responses in Mice

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The effects of xenoestrogens have been extensively studied in rodents, generally under single, high-dose conditions. Using a continuous-release, low-dose system in ovariectomized mice, we correlated the estrogenic end points of uterine epithelial height (UEH) and vaginal epithelial thickness (VET) with concentrations of two organochlorine pesticide isomers in fat and blood. Silastic capsules containing a range of doses of either β-hexachlorocyclohexane (β-HCH) or *o,p´*-dichlorodiphenyltrichloroethane (*o,p´*-DDT) were implanted subcutaneously, and animals were killed after 1 week. Average blood levels achieved by the various doses were 4.2–620 ng/mL for *o,p´*-DDT and 5.0–300 ng/mL for β-HCH. Fat concentrations of *o,p´*-DDT and β-HCH correlated linearly to blood levels (*o,p´*-DDT, r^2 = 0.94; β-HCH, r^2 = 0.83). Fat concentrations (nanograms per gram of tissue) were higher than blood concentrations (nanograms per milliliter) by 90 ± 5- and 120 ± 9-fold (mean ± SE) for *o,p´*-DDT and β-HCH, respectively. The VET ranged from 12 ± 0.9 µm in controls to 114 ± 8 µm in treated animals, and was correlated to blood levels of either treatment compound. The UEH ranged from an average of 7.7 ± 0.3 µm in controls to 26 ± 2 µm in high-dose *o,p´*-DDT-treated animals. The UEH was also correlated with β-HCH concentration, but it plateaued at approximately 11 µm at the highest doses. The lowest blood concentrations that produced statistically significant increases in VET or UEH were 18 ± 2 ng/mL *o,p´*-DDT and 42 ± 4 ng/mL β-HCH. These values are within the same order of magnitude of blood concentrations found in some human subjects from the general population, suggesting that human blood concentrations of these organochlorines may reach estrogenic levels. *Key words*: β-HCH, dose response, histology, mouse, *o,p´*-DDT, uterine epithelium, vaginal epithelium, xenoestrogen. *Environ Health Perspect* 108:973–977 (2000). [Online 7 September 2000] *http://ehpnet1.niehs.nih.gov/docs/2000/108p973-977ulrich/abstract.html*

Although banned in most industrialized nations, organochlorine (OC) pesticides are still used in Third World countries and are ubiquitous and persistent pollutants (*1*). Dichlorodiphenyltrichloroethane (DDT) is infamous as an endocrine disruptor in birds (*2*), and the *o,p´*- isomer of DDT, which constitutes approximately 15% of the technical mixture, is estrogenic *in vitro* and *in vivo* (*3*). Another estrogenic pesticide residue is the β-isomer of hexachlorocyclohexane (HCH), which constitutes 7–12% of technical HCH (*4*). β-HCH has several toxicologic effects in addition to estrogenic activity; also, it preferentially accumulates in the fat of biota and biomagnifies as it moves through the food web (*4*). Because virtually all humans have been exposed to these compounds, the concern over their potential health effects continues to grow. Recent epidemiologic studies have been controversial regarding the relationship of OC blood levels and human disease (*5–16*). The debate is likely to continue because there are no "unexposed" cohorts for comparison, and exposure information is difficult to obtain. For these reasons, we must rely on experimental animal studies as a guide to determine rational safety levels for humans.

Many studies, both *in vivo* and *in vitro*, probe a variety of effects caused by these OC pesticides. Although β-HCH does not competitively bind to the estrogen receptor (*17,18*), it does produce a number of estrogen-like responses: it stimulates proliferation and increases synthesis of progesterone receptors in cultures of human breast cancer cells (*17,18*), and it produces moderate uterotrophic effects in the rodent uterus (*18,19*). In contrast, *o,p´*-DDT does competitively bind to the estrogen receptor (*18,20,21*), and it produces a range of estrogenic responses (*18–23*). However, previous *in vivo* studies have used only a few or very high doses of these chemicals, and only the doses applied, not the resulting blood concentrations, were reported.

The present report describes an *in vivo* experiment designed to correlate the estrogenic end points of uterine epithelial cell height (UEH) and vaginal epithelial thickness (VET) with blood and fat levels of *o,p´*-DDT and β-HCH in ovariectomized mice. To simulate the effect of chronic low-level exposure seen in humans, a low dose, continuous release system of Silastic capsules was employed. A wide range of doses was administered, and concentrations of treatment compound were measured in the blood and fat of the mice. This is the first study to correlate blood and tissue concentrations of these compounds to estrogenic response in laboratory animals. These results are important to the discussion of safe exposure for humans and wildlife because they allow direct comparisons between blood levels and biological responses.

Materials and Methods

Mouse treatment. All procedures performed on animals followed the NIH *Guide for the Care and Use of Laboratory Animals* (*24*), and were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine. Three weeks after adult female CD-1 outbred mice were ovariectomized, animals were treated with *o,p´*-DDT or β-HCH over a dose range of approximately 32-fold by subcutaneous insertion of Silastic capsules containing crystalline treatment compound. A positive control group consisted of animals that received a Silastic capsule containing 20 mg estrone (Sigma Chemical Co., St. Louis, MO). Negative control animals were treated with an empty capsule. Treatment groups consisted of five animals per dose. Treatment capsules were made from Silastic tubing (1.6 mm i.d., 3.2 mm o.d., and 14 mm length; Konigsberg Instruments, Pasadena, CA) and sealed at each end with Silastic cement. Each capsule contained approximately 20 mg crystalline material. Low doses were achieved by inserting a single capsule containing treatment compound mixed with crystalline cholesterol; mixtures were prepared to make "dilutions" of one-half, one-fourth, and one-eighth. The higher doses were achieved by inserting two capsules of the one-half dilution, or one, two, or four capsules containing only test compound. Each Silastic capsule was implanted subcutaneously through a 5-mm slit on the back. After 1 week of treatment, animals were anesthetized and exsanguinated by heart puncture. The uterus and vagina of each animal was removed for histomorphometric determination of the estrogenic effect. Blood serum and intraperitoneal fat samples were

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frozen and saved for analysis of *o,p´*-DDT or β-HCH concentrations.

Histomorphometrics. Uterine and vaginal tissues were fixed in neutral formalin and processed for paraffin sections. Tissue sections (6 µm) were stained with hematoxylin and eosin. Cross-sections were examined under a light microscope (Nikon Optophot; Nikon, Fryer Co., Huntley, IL) that was interfaced with a Macintosh PowerPC computer (Apple Computer, Inc., Cupertino, CA) through a Sony 3CCD color video camera (Sony, The Systems Group, Ann Arbor, MI). The height of the uterine epithelium and the thickness of the vaginal epithelium were determined using an image analysis program (IPLab Spectrum, Signal Analytics, Vienna, VA).

Blood extractions. Three or four of the five blood samples from each dose were processed for *o,p´*-DDT or β-HCH concentration analysis. Approximately 100 µL mouse serum was extracted three times with 20 mL hexane (EM Scientific, Gibbstown, NJ). To increase the volume of the aqueous phase, 1 mL of 90% formic acid (Fisher Scientific, Fair Lawn, NJ) was added to the serum. We used γ-HCH as an internal standard for the mice treated with β-HCH and *p,p´*-DDT for the mice treated with *o,p´*-DDT (Accustandard, New Haven, CT). The resulting aqueous phase was extracted with 1 min of vortex mixing and 1 min of centrifugation for each aliquot of hexane.

Fat extractions. Fat tissues (0.25 g) were ground with 15 g of 10–60 mesh anhydrous sodium sulfate (Fisher Scientific) and spiked with the appropriate internal standard as above. These mixtures were then Soxhlet extracted for 24 hr in 300 mL 50% acetone in hexane (EM Scientific).

Quality control. With each set of six samples, we also extracted a matrix spike and either a matrix blank or a glassware blank. All quality control samples underwent the same cleanup procedure as the samples. The matrix spike contained a known amount of the target analyte similar in concentration to what we expected in the samples. Spike recovery averaged 104 ± 7%, and sample concentrations were not corrected for recovery. No target compound was found in any type of blank; therefore, blank correction was unnecessary.

Lipid analysis and removal. For fat samples only, lipid analysis was performed gravimetrically in duplicate. The removal of lipids was performed using a gel permeation chromatography column. The glass column (2.5 cm × 100 cm) was packed with SX8 Bio-Beads (Bio-Rad Laboratories, Hercules, CA) and eluted with 60% cyclohexane in dichloromethane (EM Scientific) at a flow rate of 10 mL/min through the column. The lipids were eluted in the first 200 mL fraction, whereas the HCHs and DDTs were eluted in the following 400 mL fraction.

Silica column cleanup. Sample extracts were reduced to approximately 1 mL by rotary evaporation and were exchanged into hexane as necessary. Fractionation was performed to remove possible interferences from the samples. The extracts were run through a silica (grade 923; Grace Davison, Baltimore, MD) column (1.25 cm diameter) consisting of glass wool, 20 cm of silica (1% HPLC-grade water deactivated), and 1 cm sodium sulfate. For both compounds, we collected three fractions of 75 mL each. HCH samples were fractionated with hexane, 50% dichloromethane in hexane, and dichloromethane, whereas DDT samples were fractionated with

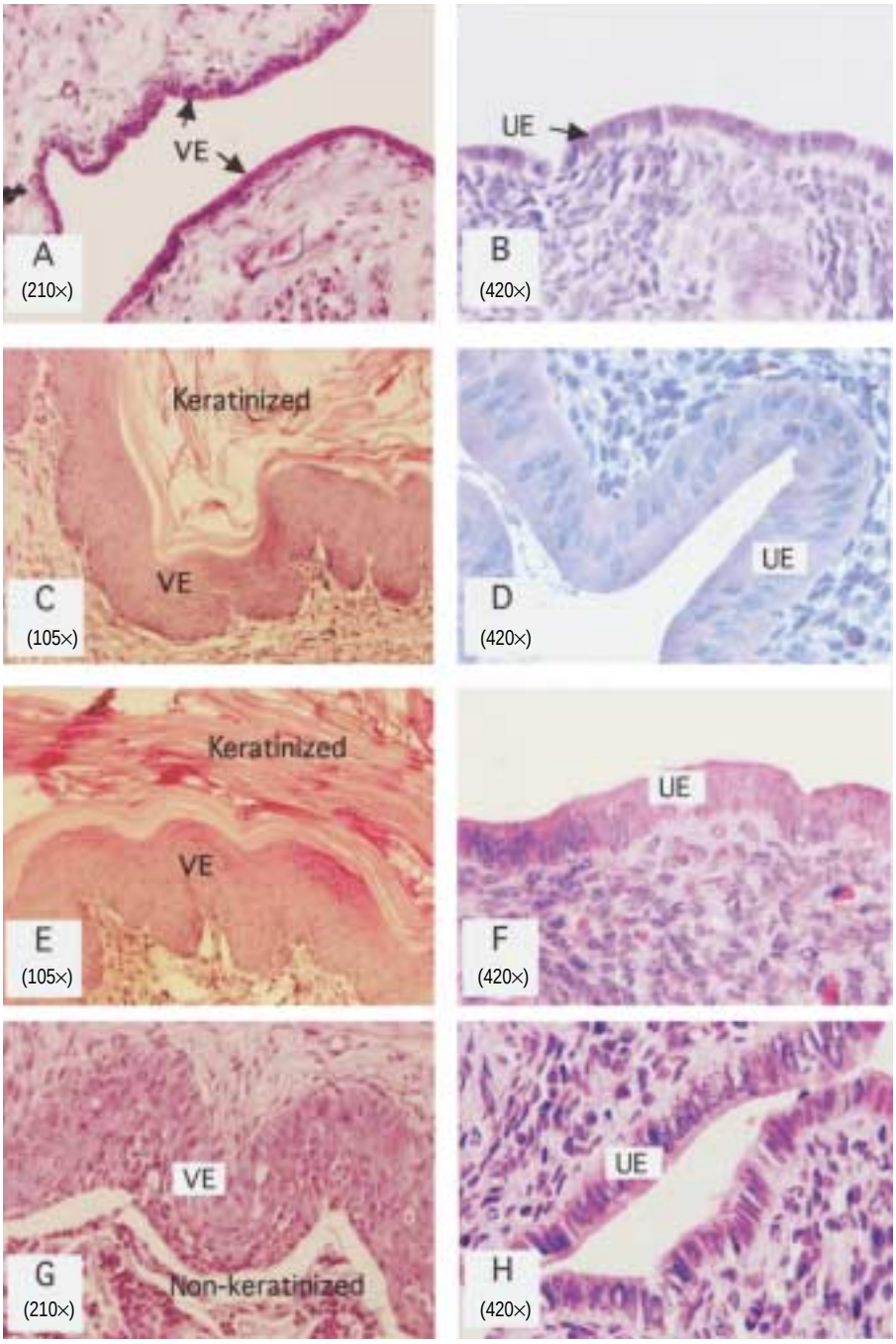

Figure 1. Histology of vagina and uterus in treated mice. Shown are representative histologic sections of vagina (*A*, *C*, *E*, *G*) and uterus (*B, D, F, H*) from mice treated for 1 week with an empty implant (*A, B*), an estrone implant (*C, D*), the highest dose of *o,p´*-DDT (*E, F*), or the highest dose of β-HCH (*G, H*). The final magnification is shown in each panel. Darker cells labeled VE and UE are the measured vaginal epithelium and uterine epithelium, respectively. In the estrone-treated (*C*) and *o,p'*-DDT-treated animals (*E*), there were several layers of keratinized cells in the superficial layers of the vaginal epithelium.

hexane, 20% dichloromethane in hexane, and dichloromethane. The HCHs and DDTs were eluted in the second fraction. All three fractions were reduced by rotary evaporation and solvent exchanged into hexane, if necessary. The first and third fractions were frozen and stored, but not analyzed. The second fraction was further reduced to approximately 50 µL by a gentle stream of nitrogen and transferred into an autosampler vial with two to three rinses of hexane.

Instrumental parameters. The samples were analyzed on a Hewlett Packard 5890A gas chromatograph (Hewlett Packard, Palo Alto, CA) with an electron capture detector. The carrier gas was hydrogen (80 mL/min split vent, 2 mL/min on column) and the makeup gas (25 mL/min) was nitrogen (Gas Tech, Hillside, IL). Injections of 1 µL were made by an autosampler in the splitless mode, and the purge flow (2 mL/min) was opened after 3 min. We used a 60-m DB5 column (J&W Scientific, Folsom, CA), with 250 µm i.d. and a film thickness of 0.1 µm, for separation. The temperature program for the DDTs was 40°C for 1 min, ramped to 130°C at 30°C/min, ramped to 241°C at 3°C/min, and ramped to 285°C at 30°C/min, with a 10-min hold for a total analysis time of 52 min. The HCH temperature program was 50°C for 1 min, ramped to 130°C at 20°C/min, ramped to 160°C at 1°C/min, and ramped to 290°C at 30°C/min, with a 1-min hold for a total analysis time of 40 min.

Statistical analysis. We used linear regression analysis to compare blood and fat concentrations of a compound to each other and to compare biological response (VET or UEH) against blood or fat concentrations of a test compound. We used analysis of variance (ANOVA) and the Dunnett Test to evaluate responses at each dose relative to control (empty capsule treatment) response values. Because variance increased with increasing dosage, response values were log transformed before applying ANOVA.

Results

Estrogenic responses. The effects of the highest doses of each compound are shown in Figure 1. *o,p´*-DDT increased VET to the same extent as estrone. In addition, the superficial layers of the vaginal epithelium were keratinized in animals treated with either estrone or *o,p´*-DDT. Uterine epithelial cell height was also increased maximally by *o,p´*-DDT. The effects of β-HCH were less than those produced by estrone. The highest dose of β-HCH produced keratinized vaginal epithelium in only two of the five animals in this group. However, there was an apparent proliferative effect in the other three animals, as evidenced by the increased tissue thickness.

Blood and fat concentrations. Blood concentrations of β-HCH and *o,p´*-DDT averaged from 5.0 to 300 ng/mL and 4.2 to 620 ng/mL, respectively. Concentrations of these compounds were much higher in fat, 1,300–42,000 ng/g tissue and 270–77,000 ng/g tissue for β-HCH and *o,p´*-DDT, respectively. Figure 2 shows the strong linear relationship between blood and fat concentrations for each compound, with fat concentrations related to blood levels by coefficients of 90 ± 5 and 120 ± 9 (SE) for *o,p´*-DDT and β-HCH (r^2= 0.94 and r^2 = 0.83), respectively.

Histomorphometrics. Control animals had VET and UEH values of 11.7 ± 0.94 µm and 7.7 ± 0.32 µm (mean ± SE), respectively. VET was increased 10-fold by the highest dose of *o,p´*-DDT and 5-fold by β-HCH at its highest dose; however, the high dose of β-HCH resulted in blood levels that were approximately one-half those achieved by the high dose of *o,p´*-DDT (Table 1). Thus, on a nanogram per milliliter basis, *o,p´*-DDT and β-HCH were equally effective in vaginal epithelium. There was a linear correlation between log blood concentration of either compound and log VET or log UEH (Figure 3A-D); in each case the correlation was statistically significant ($p \leq 0.002$ for VET and UEH against either *o,p´*-DDT or β-HCH). As expected from the strong correlation between blood and fat concentrations, dose–response curves for VET and UEH showed similar trends when plotted against fat levels (not shown). Thus, only the blood values are used for further discussions of estrogenic responses.

For the purpose of comparison, animals dosed with estrone had a VET (mean ± SE) of 80 ± 7 µm and a UEH of 25 ± 3 µm (Table 1). Mice with *o,p´*-DDT blood concentrations > 260 ng/mL had VET and UEH responses as high or higher than the estrone-induced effect. None of the β-HCH treatments caused responses as high as those achieved in estrone-treated animals; in fact, the UEH response to β-HCH reached a

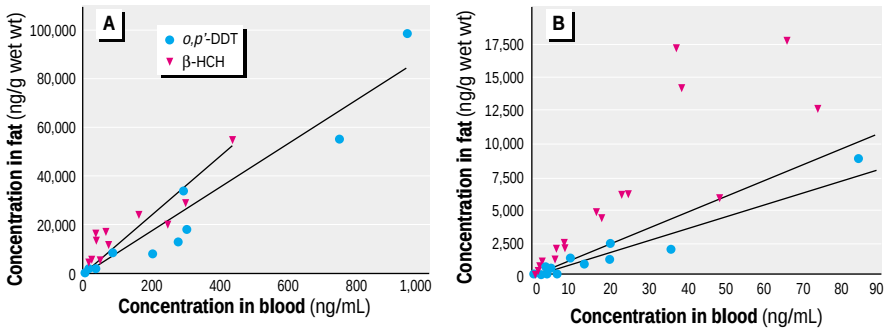

Figure 2. *o,p´*-DDT and β-HCH concentrations in fat and blood of (*A*) 20,000–100,000 ng/g wet wt and 0–1,000 ng/mL, respectively, and (*B*) 2,500–17,500 ng/g wet wt and 0–90 ng/mL, respectively. Linear relationships were forced through the origin. The slope of the line (ng/g fat)/(ng/mL blood) indicates a magnification factor from blood to fat of 120 ± 9 for β-HCH and 90 ± 5 for *o,p´*-DDT (mean ± SE). Correlation coefficients were statistically significant for both β-HCH (r^2 = 0.83; $p < 0.001$) and *o,p´*-DDT (r^2 = 0.94; $p < 0.001$).

Table 1. Blood concentrations and biological end points.

Treatment group	Blood concentration (ng/mL)	UEH (µm)	VET (µm)
Control	0	7.74 ± 0.32 (5)	11.7 ± 0.94 (5)
Estrone	ND	25.5 ± 3.5 (5)**	79.6 ± 6.6 (5)**
o,p´-DDT			
A (1/8)	4.4 ± 0.61 (4)	7.88 ± 0.62 (4)	14.3 ± 2.5 (4)
B (1/4)	5.2 ± 0.91 (3)	8.93 ± 0.22 (3)	14.7 ± 0.87 (3)
C (1/2)	23 ± 13 (2)	9.15 ± 0.66 (3)	20.0 ± 1.7 (4)*
D (2 × 1/2)	18 ± 2.3 (3)	12.0 ± 0.95 (5)**	39.7 ± 7.0 (5)**
E (1)	190 ± 40 (4)	11.4 ± 1.6 (4)*	63.9 ± 10 (4)**
F (2)	260 ± 26 (4)	15.7 ± 1.9 (4)**	85.3 ± 21 (4)**
G (4)	620 ± 150 (4)	26.0 ± 1.7 (4)**	114 ± 8.4 (4)**
β-HCH			
A (1/8)	5.0 ± 0.97 (3)	8.40 ± 0.61 (4)	12.1 ± 1.7 (4)
B (1/4)	12 ± 3.4 (3)	7.18 ± 0.75 (4)	15.1 ± 1.1 (4)
C (1/2)	22 ± 2.5 (3)	9.03 ± 1.2 (5)	17.6 ± 4.2 (5)
D (2 × 1/2)	42 ± 3.6 (3)	9.97 ± 1.0 (4)	21.9 ± 2.7 (5)**
E (1)	66 ± 3.9 (3)	12.1 ± 0.61 (4)**	38.3 ± 6.1 (4)**
F (2)	170 ± 29 (4)	10.8 ± 0.71 (5)*	38.9 ± 2.9 (5)**
G (4)	300 ± 49 (4)	11.3 ± 0.82 (5)*	58.1 ± 5.2 (5)**

ND, not determined. Mean values of blood serum concentrations, UEH, and VET were determined for each treatment/dosage group. For *o,p´*-DDT and β-HCH, dosage groups are listed as A–G for treatments with a single diluted capsule (1/8, 1/4, 1/2), a single undiluted capsule (1), or multiple capsules (2 × 1/2, 2, 4). Values are presented as mean ± SE; numbers in parentheses are the number of samples analyzed.
*$p < 0.05$. **$p < 0.005$ as compared to control.

plateau of approximately 11 µm for blood concentrations > 66 ng/mL (Table 1).

The doses in this experiment are low enough to observe a no-observed-effect level (NOEL). The lowest-observed-effect levels (LOELs) in VET were detected at blood concentrations of 42 ng/mL and 18 ng/mL for β-HCH and *o,p´*-DDT, respectively (Table 1). Statistically significant increases in UEH were detected at blood concentrations of 66 ng/mL and 18 ng/mL for β-HCH and *o,p´*-DDT, respectively (Table 1).

Discussion

Blood and fat concentrations. There is a strong linear relationship between the blood and fat concentrations for both compounds. For β-HCH, fat levels (nanograms per gram wet wt) were related to blood levels (nanograms per milliliter) by a factor of 120; fat levels of *o,p´*-DDT were related to blood levels by a factor of 90. Similar trends have been observed in humans. In a Canadian study of paired blood and fat concentrations, Mes (*25*) found 43- to 355-fold higher levels of β-HCH in fat and a 33-fold higher level of *o,p´*-DDT in fat. In an Indian study, Ramachandran et al. (*26*) found 35-fold (male) to 38-fold (female) higher levels of β-HCH in fat and 20-fold (female) to 25-fold (male) higher levels of *o,p´*-DDT in fat. The linearity of these relationships allows us to use either blood or fat values in our discussion of estrogenic effects. Because blood levels are more readily available in reports of human exposure, we will focus on the blood levels attained in our experimental system.

Dose–response curves. In general, β-HCH produced a slightly lower response than did *o,p´*-DDT across all blood levels observed. There is a marked difference in the maximal efficacy of these compounds, especially in the uterus. In both the uterus and vagina, *o,p´*-DDT is more potent than β-HCH, producing a significant effect at 18 ng/mL compared to 42 ng/mL required for a significant effect by β-HCH. The observation that the uterine response to β-HCH reached a plateau at higher doses suggests that β-HCH is a compound with differential effects of the vagina and uterus. Nishino and Neumann (*27*) have shown that two estrogen derivatives [8α-estra-1,3,5(10)-triene-1,3,17β-triol and 1,3,17β-triacetoxy-8α-estra-1,3,5(10)-triene] produced estrogenic effects in the mouse vagina, but not in the uterus. It may be that β-HCH also behaves in a selective manner, having qualitatively different effects in the vagina and uterus.

Concentration comparison to humans. The biological response to the low levels of these compounds was unexpected. Table 2 shows that the concentrations of OC pesticides in different unexposed human populations were generally only 2.7–120 (*o,p´*-DDT) or 2–140 (β-HCH) times lower than concentrations found to cause estrogenic responses in mice. However, in Israel, *o,p´*-DDT blood concentrations were found to be as high as 32 ng/mL (*28*), nearly double the minimal estrogenic blood level of 18 ng/mL observed in this study. Argentinean pesticide workers had blood β-HCH concentrations as high as 240 ng/mL (*29*); this is nearly six times the minimal estrogenic level of 42 ng/mL found in this study.

Although it is difficult to determine if the response in humans is the same as in mice, it is alarming that the human blood concentrations are so similar to the estrogenic concentrations in mice. The mouse has proven to be a good model for approximating estrogenicity of a compound in humans. For example, although the antiestrogens tamoxifen and clomiphene show little estrogenicity in the standard uterine weight and vaginal cornification bioassays performed in the rat, they are fully estrogenic in the mouse (*30–33*), and these compounds behave as estrogen agonists in the human uterus and vagina (*34–36*). Also, the mouse model of developmental effects of estrogens in the female and male reproductive tracts accurately reflects the effects that diethylstilbestrol had on developing human reproductive

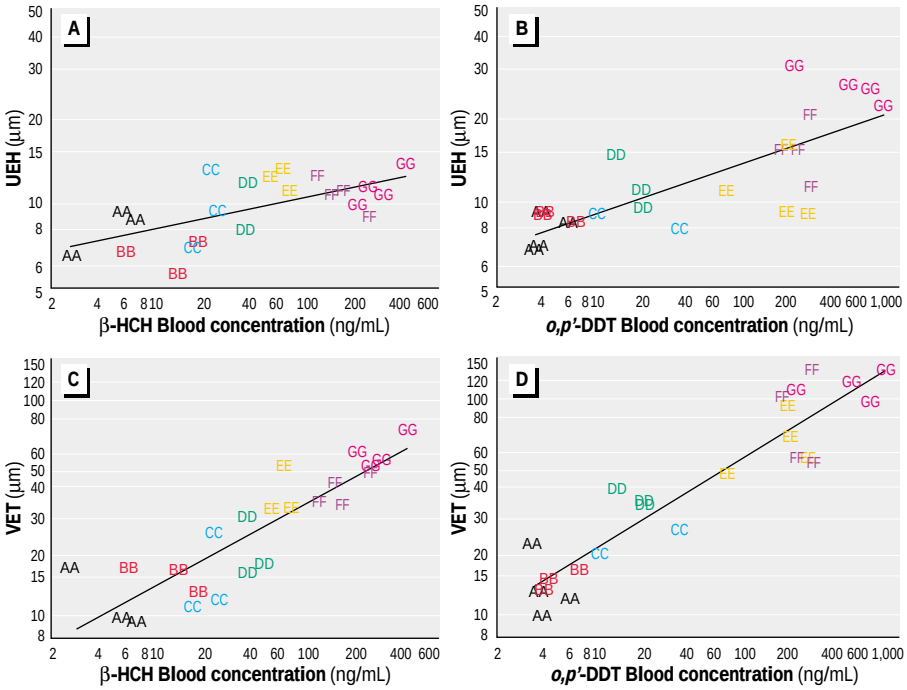

Figure 3. UEH and VET dose–response curves for β-HCH (*A* and *C*, respectively) and *o,p´*-DDT (*B* and *D*, respectively). Samples for which there were paired response and blood concentration values for β-HCH or *o,p´*-DDT were used in log–log plots. Individual samples from the different dosages (1/8, 1/4, 1/2 … 4 capsules) are shown with letters AA, BB, CC … GG, respectively, (compare treatment groups listed in Table 1). The square of the correlation coefficient, r^2, for UEH was 0.39 for β-HCH ($p < 0.002$) (*A*) and 0.58 for *o,p´*-DDT ($p < 0.0001$) (*B*). The r^2 for VET was 0.75 for β-HCH ($p < 0.0001$) (*C*) and 0.87 for *o,p´*-DDT ($p < 0.0001$) (*D*).

Table 2. Concentrations of β-HCH and *o,p´*-DDT reported in human blood.

Sample description	β-HCH (ng/mL)	*o,p´*-DDT (ng/mL)
Canada 1992[a] (*25*)	ND–2.6	ND–0.27
India male 1982 (*26*)	0.30 ± 0.03	0.15 ± 0.02
Pakistan 1987 (*28*)	0–7.2	
Yugoslavia 1985–1986 (*28*)	1.7	
Israel 1984–1985 (*28*)		ND–32
United States (NOE) 1971[a] (*29*)	1.4 ± 0.46	
Argentina (NOE) 1971[a] (*29*)	23 ± 10	0.27 ± 0.32
Argentina pesticide workers 1971[a] (*29*)	240 ± 140	
Japan 1986–1988 (*46*)	0.7–5.5	
England 1967 (*47*)	4.1	
Japanese in Florida (USA) 1971[a] (*48*)	14–17	1.5–2.2
Taiwanese in Florida (USA) 1971[a] (*48*)	3.6–21	Trace–6.6
Florida (USA) 1971[a] (*48*)	0.4 ± 2.0	0.7–2.0
India (NOE) 1992 (*49*)	13 ± 5.0	
India pesticide workers 1992 (*49*)	33 ± 23	1.5 ± 4.5
Mice with estrogenic response (this study)	42 ± 3.6	18 ± 2.3

Abbreviations: ND, not detected (or below detection limit of the assay used); NOE, nonoccupationally exposed.
[a]No sampling date was listed; therefore, the year of publication is given.

tracts (*37–39*), whereas the tests performed in a primate model were uninformative (*40*). On the other hand, the choice of the outbred CD-1 mouse may not have been optimum for determination of estrogen sensitivity; Spearow et al. (*41*) found that other strains are much more sensitive to the effects of estradiol in the developing testes. Similarly, we have found that strain differences can affect the outcome of the rat vaginal bioassay for xenoestrogen activity (*42*). Which strain of mouse is most appropriate for comparison to the human situation is not known, but certainly the results of the present study indicate that blood levels of *o,p´*-DDT or β-HCH in the nanograms per milliliter range have the potential of producing estrogenic effects.

This study was designed to produce blood and tissue levels of *o,p´*-DDT or β-HCH in mice comparable to those found in people from the general population, that is, those who are not exposed through a job-related or accidental event. Humans are exposed to OCs on a daily basis through respiration, foods, and contact (*16*). Additionally, circulating levels may increase during periods of weight reduction. As is generally seen in human studies, (*25*, *26*), the concentration of OC pesticides is much higher in fat stores. Fat concentration is in equilibrium with blood concentration; thus, during periods of decreasing weight and fat stores, the compounds in fat are released into the blood stream and surrounding tissues (*43,44*). Bigsby et al. (*19*) showed that this OC release from fat can cause estrogenic responses in mice. This is especially important in fatty areas like the breast, in which the parenchyma is the target of estrogenic compounds that may act as tumor promoters (*16,45*).

This study is the first to measure tissue or blood levels of OC pesticide concentrations and correlate them with estrogenic responses in a laboratory animal. The extremely low levels required to cause statistically significant effects compared to control animals were unexpected. It is even more alarming that there is little difference between these levels and those that can be found in humans. Future studies that include long-term, low-dose exposure are required to simulate the chronic low-level exposure seen in humans.

References and Notes

1. Simonich SL, Hites RA. Global distribution of persistent organochlorine compounds. Science 269:1851–1854 (1995).
2. Fry DM. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ Health Perspect 103:(suppl 7):165–171 (1995).
3. Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 103:(suppl 7):113–122 (1995)
4. Willett KL, Ulrich EM, Hites RA. Differential toxicity and environmental fates of hexachlorocyclohexane isomers. Environ Sci Technol 32:2197–2207 (1998).
5. Dewailly É, Dodin S, Verreault R, Ayotte P, Sauvé L, Morin J, Brisson J. High organochlorine body burden in women with estrogen receptor-positive breast cancer. J Natl Cancer Inst 86:232–234 (1994).
6. Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J Natl Cancer Inst 86:589–599 (1994).
7. MacMahon B. Pesticide residues and breast cancer [Editorial]. J Natl Cancer Inst 86:572–573 (1994).
8. Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. J Natl Cancer Inst 85:648–652 (1993).
9. Perlmutter D. Organochlorines, breast cancer, and GATT. JAMA 271:1160–1161 (1994).
10. Høyer AP, Grandjean P, Jørgensen T, Brock JW, Hartvig HB. Organochlorine exposure and risk of breast cancer. Lancet 352:1816–1820 (1998).
11. Hunter DJ, Hankinson SE, Laden F, Colditz GA, Manson JE, Willett WC, Speizer FE, Wolff MS. Plasma organochlorine levels and the risk of breast cancer. N Engl J Med 337:1253–1258 (1997).
12. López-Carrillo, L, Torres-Arreola L, Torres-Sanchez L, Espinosa-Torres F, Jiménez C, Cebrián M, Waliszewski S, Saldate O. Is DDT use a public health problem in Mexico? Environ Health Perspect 104(suppl 6):584–588 (1996).
13. Moysich KB, Ambrosone CB, Vena JE, Shields PG, Mendola P, Kostyniak P, Greizerstein H, Graham S, Marshall JR, Schisterman EF, et al. Environmental organochlorine exposure and postmenopausal breast cancer risk. Cancer Epidemiol Biomark Prev 7:181–188 (1998).
14. Hoffmann W. Organochlorine compounds: risk of non-Hodgkin's lymphoma and breast cancer? Arch Environ Health 51:189–192 (1996).
15. Olaya-Contreras P, Rodr'guez-Villamil J, Posso-Valencia, HJ, Cortez JE. Organochlorine exposure and breast cancer risk in Colombian women. Cad Saúde Pública 14(suppl 3):125–132 (1998).
16. Adami HO, Lipworth L, Titus-Ernstoff L, Hsieh CC, Hanberg A, Ahlborg U, Baron J, Trichopoulos D. Organochlorine compounds and estrogen-related cancers in women. Cancer Causes Control 6:551–566 (1995).
17. Coosen R, Van Velsen FL. Effects of the β-isomer of hexachlorocyclohexane on estrogen-sensitive human mammary tumor cells. Toxicol Appl Pharmacol 101:310–318 (1989).
18. Steinmetz R, Young PCM, Caperell-Grant A, Gize EA, Madhukar BV, Ben-Jonathan N, Bigsby RM. Novel estrogenic action of the pesticide residue beta-hexachlorocyclohexane in human breast cancer cells. Cancer Res 56:5403–5409 (1996).
19. Bigsby RM, Caperell-Grant, A, Madhukar BV. Xenobiotics released from fat during fasting produce estrogenic effects in ovariectomized mice. Cancer Res 57:865–869 (1997).
20. Kupfer D, Bulger WH. Interaction of *o,p'*-DDT with the estrogen-binding protein (EBP) in human mammary and uterine tumors. Res Comm Chemical Pathol Pharmacol 16:451–462 (1977).
21. Robison AK, Sirbasku DA, Stancel GM. DDT supports the growth of an estrogen-responsive tumor. Toxicol Lett 27:109–113 (1985).
22. McBlain WA, Lewin V, Wolfe FH. Differing estrogenic activities for the enantiomers of *o,p´*-DDT in immature female rats. Can J Physiol Pharmacol 54: 629–632 (1976).
23. Robison AK, Schmid WA, Stancel GM. Estrogenic activity of DDT: estrogen-receptor profiles and the responses of individual uterine cell types following *o,p'*-DDT administration. J Toxicol Environ Health 16:493–508 (1985).
24. Institute of Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals. Washington, D.C:National Academy Press, 1996.
25. Mes J. Organochlorine residues in human blood and biopsy fat and their relationship. Bull Environ Contam Toxicol 48:815–820 (1992).
26. Ramachandran M, Banerjee BD, Gulati M, Grover A, Zaidi SSA, Hussain QZ. DDT and HCH residues in the body fat and blood samples from some Delhi hospitals. Indian J Med Res 80:590–593 (1984).
27. Nishino Y, Neumann F. 8α-Estra-1,3,5(10)-triene-1,3,17β-triol as a special type of estrogen having a high vaginotrophic activity and a low uterotrophic activity in castrated mice. Steroids 30:179–192 (1977).
28. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr, Jegou B, Jensen TK, Jouannet P, Keiding N, et al. Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(suppl 4):741–803 (1996).
29. Radomski JL, Astolfi E, Deichmann WB, Rey AA. Blood levels of organochlorine pesticides in Argentina: occupationally and nonoccupationally exposed adults, children and newborn infants. Toxicol Appl Pharmacol 20:186–193 (1971).
30. Clitheroe HJ, Bonnycastle DD, Kukla L. Effects of clomiphene citrate on the mouse uterus. Proc Soc Exp Biol Med 122:70–73 (1966).
31. Harper MJ, Walpole AL. A new derivative of triphenylethylene: effect on implantation and mode of action in rats. J Reprod Fertil 13:101–119 (1967).
32. Martin L. Estrogens, anti-estrogens and the regulation of cell proliferation in the female reproductive tract *in vivo*. In: Estrogens in the Environment (Mclachlan JA, ed). New York:Elsevier/North Holland, 1980;103–129.
33. O'Connor JC, Cook JC, Craven SC, Van Pelt CS, Obourn JD. An *in vivo* battery for identifying endocrine modulators that are estrogenic or dopamine regulators. Fundam Appl Toxicol 33:182–195 (1996).
34. Cunha GR, Taguchi O, Namikawa R, Nishizuka Y, Robboy SJ. Teratogenic effects of clomiphene, tamoxifen, and diethylstilbestrol on the developing human female genital tract. Hum Pathol 18:1132–1143 (1987).
35. Fornander T, Rutqvist LE, Wilking N. Effects of tamoxifen on the female genital tract. Ann New York Acad Sci 622:469–476 (1991).
36. Kedar RP, Bourne TH, Powles TJ, Collins WP, Ashley SE, Cosgrove DO, Campbell S. Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomised breast cancer prevention trial. Lancet 343:1318–1321 (1994).
37. Bern HA, Talamantes FJ. Neonatal mouse models and their relation to disease in the human female. In: Developmental Effects of Diethylstilbestrol (DES) in Pregnancy (Herbst AL, Bern WA, eds). New York:Thieme-Stratton, 1981;29–147.
38. McLachlan JA, Newbold RR. Estrogens and development. Environ Health Perspect 75:25–27 (1987).
39. Newbold R. Cellular and molecular effects of developmental exposure to diethylstilbestrol: implications for other environmental estrogens. Environ Health Perspect 103:(suppl 7):83–87 (1995).
40. Hertz R. The estrogen problem: retrospect and prospect, In: Estrogen in the Environment II (McLachlan JA, ed). New York:Elsevier, 1985;1–14.
41. Spearow JL, Doemeny P, Sera R, Leffler R, Barkley M. Genetic variation in susceptibility to endocrine disruption by estrogen in mice. Science 285:1259–1261 (1999).
42. Long X, Steinmetz R, Ben-Jonathan N, Caperell-Grant A, Young PCM, Nephew KP, Bigsby RM. Strain differences in vaginal responses to the xenoestrogen bisphenol A. Environ Health Perspect 108:243–247 (2000).
43. Dale WE, Gaines TB, Hayes WJ Jr. Storage and excretion of DDT in starved rats. Toxicol Appl Pharmacol 4:89–106 (1962).
44. Lakshmanan FL, Pommer A, Patterson O. Chlorinated hydrocarbon insecticide residues in tissues of rats before and after reduction of body fat by dietary restriction. J Agric Food Chem 27:720–725 (1979).
45. Wassermann M, Nogueira DP, Tomatis L, Mirra AP, Shibata H, Arie G, Cucos S, Wassermann D. Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. Bull Environ Contam Toxicol 15:478–484 (1976).
46. Sasaki K, Ishizaka T, Suzuki T, Takeda M, Uchiyama M. Accumulation levels of organochlorine pesticides in human adipose tissue and blood. Bull Environ Contam Toxicol 46:662–669 (1991).
47. Yeh CY, Kuo PH, Tsai ST, Wang GY, Wang YT. A study on pesticide residues in umbilical cord blood and maternal milk. J Formos Med Assoc 75:463–470 (1976).
48. Radomski JL, Deichmann WB, Rey AA, Merkin T. Human pesticide blood levels as a measure of body burden and pesticide exposure. Toxicol Appl Pharmacol 20:175–185 (1971).
49. Dua VK, Pant CS, Sharma VP, Pathak GK. Determination of HCH and DDT in finger-prick whole blood dried on filter paper and its field application for monitoring concentrations in blood. Bull Environ Contam Toxicol 56:50–57 (1996).