# ENVIRONMENTAL ESTROGENS: POTENTIAL FOR REPRODUCTIVE TOXICITY IN FISH

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## INTRODUCTION

Anthropogenic chemicals may adversely affect populations via several mechanisms. Interference with biological systems at the molecular level can have far-reaching consequences at the organismal and population level. Chemicals that disrupt the endocrine system have negative results on the population by virtue of developmental and reproductive impairment. One class of these endocrine disruptors are compounds that mimic or antagonize the effect of endogenous estrogens, referred to as environmental estrogens or xenoestrogens. A number of environmental contaminants have been recognized as environmental estrogens including chlorinated hydrocarbon pesticides, degradation products of high-use industrial surfactants, by-products of industrial processes such as paper pulp mills and potent synthetic estrogens used in estrogen-replacement therapy and oral contraceptive formulations which find their way to the environment through municipal sewage.

Most environmental estrogens act by a similar mechanism as estradiol (E2). E2 induces its biological effects by binding to a protein, the estrogen receptor, and this steroid:receptor complex interacts with DNA to promote protein synthesis. However, most environmental estrogens have less affinity for the estrogen receptor, thus, less potency than E2. There are also some recognized indirect mechanisms of estrogenic activity such as interference with enzymes in the steroidal metabolic pathways or interaction at points along the reproductive axis upstream of the target tissue (*e.g.*, hypothalamus or pituitary). Considering the role of sex steroids in the regulation of reproductive processes and development of reproductively competent organisms, xenoestrogens have the potential to be devastating to populations. In mammals, environmental estrogenic activity has been linked to reproductive disorder and disruption of developmental processes subsequent to exposure.

Clearly, the survival of a species depends on successful development and reproduction. Reproductive toxicity may occur during larval development, or, because many of these compounds are designed to be resistant to degradation and metabolism, they can be bioaccumulated and may compromise the successful maturation of the gonads in a later stage. This investigation describes the first year of a project, supported by the Water Resources Research Institute, in which the investigators sought to identify estrogenic compounds in channel catfish based on their ability to induce vitellogenin (Vg). Vg is a glycophospholipoprotein which serves as yolk precursor and is taken up by the ovary from the blood where it is incorporated into the developing oocytes and cleaved into yolk proteins. The overall research strategy is designed to test the hypothesis that the estrogenic activity of select chemicals results in reproductive toxicity.

## MATERIALS AND METHODS

### Animal Model

Channel catfish (*Ictalurus punctatus*) were chosen as the animal model because this fish is an important aquaculture crop in the Mississippi Delta; there are several sources of fish as well as several sources of culture advice. Sexually immature catfish were obtained from the U.S. Biological Service Fish Farming Experimental Laboratory in Stuttgart, Arkansas. Both males and females (65 to 95 g) were maintained under flow-through conditions at room temperature in either a Frigid Living Stream (acclimation) or 40 liter aquaria (exposures).

### Model Compounds

17β-Estradiol (E2), diethylstilbestrol (DES), ethinylestradiol (EE2), mestranol (ME2) and tamoxifen (TMX) were obtained from Sigma Chemical Company. E2 is the most important female sex steroid in mammals and teleosts. DES, EE2 and ME2 are synthetic estrogens and TMX is an antiestrogen; all of these are used in drug therapies. Analytical grade pesticides, *o,p'*-DDT, methoxychlor (MXC), chlordecone (CLD), lindane and β-HCH were obtained from Radian International. *p*-Nonylphenol (NP) was a gift of Schenectady International (CAS RN 84852-15-3*). These compounds were selected on the basis of their

## Vitellogenin Induction

Fish were anaesthetized using MS-222 and exposed to the model compounds with a single intraperitoneal injection of a 0.25% agar suspension on day 0. Each experiment included a negative control group, the highest volume of vehicle (10 ml/kg), and a positive control group, 0.6 mg E2/kg (ED50 for Vg production). Preliminary data collected included dose- and time-response curves for E2 to determine the duration of experiments and the ED50 dose. On day 7, blood samples were taken from the caudal vein and fish were sexed by internal examination; liver weights were recorded. The hepatic:somatic index (HSI) was determined as the ratio of liver weight:body weight.

Fish were exposed to compounds either alone (for the screening part of the study) or in combination with the ED50 dose of E2 (for the interaction part of the study). Doses administered of the synthetic estrogens DES, EE2 and ME2 were equimolar to the ED50 of E2. Preliminary exposures were used to examine the remaining potentially estrogenic compounds for indication of estrogenic activity as well as to determine the highest tolerated dose. Treatment groups in these preliminary experiments had three to four fish. If there was no Vg induction after exposure to the highest dose, these compounds were deemed void of estrogenic activity in the paradigm. The highest dose was limited by lethality or volume of injection. For compounds that appeared to have estrogenic activity in the preliminary phase, a follow-up experiment was conducted with larger sample sizes. Treatments included negative and positive control, two dose groups of the xenobiotic and two combination groups in which the fish received the 0.6 mg E2/kg simultaneously along with one of two doses of xenobiotic.

Collected blood was allowed to clot for 2 to 3 hours at 4°C and centrifuged to isolate serum. Serum samples were stored at -60°C until analysis. Serum concentrations of Vg were determined with a competitive-type enzyme-linked immunosorbent assay (ELISA) utilizing monoclonal antibodies specific for channel catfish Vg. The anti-Vg was a gift from John M. Grizzle (Auburn University); the use of the antibody in an ELISA has been described (Goodwin et al. 1992). Vg used in the assay was partially purified by precipitation with EDTA and $MgCl_2$ from the serum of E2-treated mature female catfish.

# RESULTS

## Vitellogenin Induction as an Estrogenic Screen

Dose-response and time-response curves of the induction of Vg following E2 administration were constructed to determine the optimum sampling time as well as a dose to use in subsequent experiments as a positive control. The peak of Vg appearance in serum was at 11 days, but the protein was detected within 24 hours; the ED50 for induction was 0.6 mg/kg. Figure 1 shows the dose-response nature of Vg induction following a single intraperitoneal injection of E2 as well as the HSI over the same dose range. Increases in liver weight are not significantly different from the agar vehicle control until the highest dose tested, 500 mg/kg.

Results from screening therapeutic estrogens are presented in both Figure 2 and Table 1. Vg induction is expressed as percent of the response to E2 while HSI is expressed as percent of the index in agar control fish in order to compare between experiments. EE2 and ME2 treatment resulted in both significantly higher Vg serum levels (Figure 2) and greater HSI (Table 1) than the equimolar E2 dose. On the other hand, DES induced Vg but was found to be less potent than E2 (Figure 2). DES treatment resulted in no significant change in HSI, compared to the negative agar control. Two doses of the antagonist TMX were examined: 0.82 and 136.4 mg/kg, equimolar to 0.6 and 100 mg E2/kg, respectively. Results in Figure 2 and Table 1 do not indicate an agonist (estrogenic) effect at either dose.

## Screening Xenobiotics for Estrogenic Activity

Four of the xenobiotics screened in preliminary experiments failed to induce Vg or changes in HSI, compared to negative controls. These compounds and the highest doses tested in the screening are presented in Table 2. The doses are expressed in absolute values as well as the equimolar E2 dose. Exposure to CLD and lindane was limited because doses higher than those in Table 2 resulted in lethality. The highest doses of β-HCH and *o,p'*-DDT resulted in no visible toxicity but higher doses were impractical due to limitations in injection volume and absorption from the peritoneal cavity.

Both NP and MXC demonstrated evidence of being estrogenic (Table 3). At both doses equimolar to 100 and 300 mg E2/kg, NP resulted in Vg appearance in the serum. The response was variable among individuals, therefore only the highest dose is significantly different from the agar control. Individual serum levels ranged from 0.6 to 9.9 mg/mL for the low dose and 0.1 to 14.7mg/mL for the high dose. The high dose of NP is 500 times greater than the positive control E2, yet Vg levels were more than an order of magnitude less. Vg induction by MXC was also variable, therefore induction was not different from the negative control at either dose (equimolar to 100 and 300 mg/kg) even though the range of Vg concentrations was 0.27 to 9.99 mg/mL for the low and 0.03 to 13.75 mg/mL for the high dose.

EE2, ME2 and DES (equimolar to 0.6 mg E2/kg) were co-administered with E2 (0.6 mg/kg) using the same exposure and sampling conditions as in the screening experiment. A combination dose of EE2/E2 and ME2/E2 resulted in Vg levels greater than E2 alone, but the same as EE2 and ME2 alone (Figure 3). TMX was co-administered at two doses, equimolar to 0.6 and 100 mg E2/kg with E2. At the high dose, the antiestrogen was able to inhibit the estrogenic response (Figure 3).

MXC and NP were also tested in this paradigm of combination dosing (Figure 4). Both compounds were co-administered as doses equimolar to 100 and 300 mg E2/kg with E2. Neither dose of NP changed the normal response to E2 significantly despite a trend towards less Vg. There was also a trend towards reduction of Vg over both doses of MXC but only the highest dose was significantly less than E2. This was also the only dose of MXC that demonstrated any mortality, 38% died.

## DISCUSSION

Screening known potent estrogens served as a means to validate the catfish vitellogenesis model as consistent with the estrogen-responsiveness of mammalian systems. The higher potency demonstrated by EE2 and ME2 is consistent with greater binding affinity of EE2 for the mammalian estrogen receptor, 158% that of E2 (Fritsch 1991). ME2 is a proestrogen requiring o-demethylation for biological activity in mammals; these results indicate that a similar metabolic transformation occurs in catfish. It appears that this dose of ME2 and EE2 induces the maximum vitellogenic response because when given with an equimolar dose of E2 there is no increase in Vg induction. While DES is known to be a potent nonsteroidal estrogen in mammalian systems, relative binding affinity is 141%, it was found to be less potent than E2 in these investigations. Thomas and Smith (1993) reported that DES had a similar binding affinity for the spotted seatrout liver as E2 while Pelissero et al. (1993) found that DES was less effective than E2 in stimulating rainbow trout hepatocytes to produce Vg. The DES/E2 combination dose resulted in Vg induction greater than E2 or DES alone indicating that the interaction between the two compounds is at least additive. TMX was not expected to have an estrogenic effect; this nonsteroidal triphenylethylene is classified as a partial agonist for the estrogen receptor in mammals because its action as an agonist or antagonist depends on the tissue and the presence of E2 (Jordan 1984). In the catfish model, no partial agonist activity of TMX was evident. When given in combination with E2, the high dose of TMX (equimolar to 100 mg/kg E2) was able to inhibit the vitellogenic response. Therefore, TMX would be classified as a pure antagonist in this model, with less affinity for the receptor than E2. The vitellogenic responses for DES, EE2, ME2 and TMX provides evidence for the similarity of the fish estrogenic model to that in mammals in terms of receptor mediation and binding specificity.

Four of the xenobiotics predicted to have estrogenic activity (Table 2) failed to induce Vg induction in this model. There is evidence in both the mammalian and fish literature that these compounds are estrogenic. Low, chronic administration of β-HCH has been reported to be estrogenic in medaka and guppies on the basis of histological changes associated with induced vitellogenesis (Wester et al. 1985; Wester and Canton 1986). The absence of biological activity of the DDT isomer investigated here is consistent with the results of a competitive binding study which found that *o,p'*-DDT did not bind to the estrogen receptor in seatrout liver (Thomas and Smith 1993). However, Denison et al. (1981) correlated the presence of a "Vg-like" protein identified by PAGE/Coomassie with DDT resistance in mosquitofish. An estrogenic response to the relatively nontoxic *o,p'*-DDT and β-HCH may have been noted under a chronic dosing regime. The estrogenic activity of CLD in mammalian and avian models has been well documented (see Eroschenko 1981). In addition, histologically-scored damage to testes and ovaries in freshwater catfish, *Heteropneustes fossilis*, has been found following exposure to CLD; the investigators hypothesize that toxicity may be due in part to estrogenic effects (Srivastava and Srivastava 1994). The absence of Vg induction in the catfish model following CLD exposure was most likely because the toxic acute dose resulted in lower concentration at the liver than the dose required to cause estrogenic activity. There is evidence of both estrogenic and antiestrogenic activity of lindane in rats (Raizada et al. 1980; Chadwick et al. 1988). Although there are conflicting explanations for the mechanism of action, these reports agree that lindane interferes at some point along the reproductive axis. There is no evidence in the literature indicating whether these effects are similar in fish. In the present investigation, lindane, like CLD, was more toxic than either *o,p'*-DDT or β-HCH and estrogenic activity was not evident in catfish.

NP was found to be estrogenic based on its ability to induce the appearance of Vg in serum but the increase was statistically significant at only a high dose. The low potency is consistent with that seen with mammalian systems (Soto et al. 1991), as well as *in vitro* and *in vivo* fish models (Jobling and Sumpter 1993; Lech et al. 1996). When rainbow trout hepatocytes are treated *in vitro* with NP and its related compounds (octylphenol, carboxylic acid derivatives) the potencies for Vg induction are 4 to 6 orders of magnitude less than for E2 (Jobling and Sumpter 1993). The affinity of NP for the trout estrogen receptor is 1000 times less than E2 (White et al. 1994).

MXC was shown to have a low estrogenic potency. The increase in serum Vg was not significantly different from the agar control because of individual variation. MXC has been shown in mammalian systems to be a proestrogen, requiring o-demethylation to a hydroxylated metabolite (Kupfer and Bulger 1980). Thomas and Smith (1993) reported that MXC itself is devoid of binding affinity in spotted seatrout. The individual variation found in the present study may be the result of overall lower constitutive metabolizing enzyme activity in fish compared to mammals.

One hypothesis regarding environmental estrogens is that they have the potential to interfere with normal actions of E2. In the combination experiments an attempt was made to model consequences of exposure to environmental estrogens in a vitellogenic, mature female, *i.e.*, one responding to E2 as part of the normal reproductive process. While not significant, treatment with NP decreased the vitellogenic response to E2. MXC also caused a decrease in serum Vg compared to E2 alone, however, the lethality seen at this dose makes it impossible to say with any degree of certainty whether the observed decrease in the remaining fish was due to antagonism of the response directly or through general morbidity.

While the liver in fish is the target organ of E2, increases in HSI were only observed following treatment with potent synthetic estrogens. In the dose-response curve used to establish the ED50 of E2 Vg induction, increases in the HSI were not observed until between 1 and 10 mg/kg whereas increased Vg was observed between 0.01 and 0.1 mg/kg. Therefore, the sensitivity of vitellogenesis makes it the preferred indicator of estrogenicity. Also, unlike uterine weight in mammals, changes in fish liver weight may be indicative of biochemical adaptations other than interaction with the estrogen receptor, *i.e.*, hypertrophy accompanying induction of metabolizing enzymes.

The *in vivo* vitellogenesis paradigm for estrogen receptor activation in channel catfish appears to be consistent with mammalian literature when synthetic estrogens and antiestrogens are tested. The estrogenicity associated with NP and MXC was demonstrated by the acute induction of vitellogenesis following intraperitoneal injection. However, the present *in vivo* screening assay has limitations. There is wide variation in the vitellogenic response in individual fish to both xenoestrogens and E2. These differences do not appear to be due to gender or size (within the established range), but sample sizes were not large enough to permit this kind of analysis. In addition, as demonstrated by CLD, the acute, single dose of environmental contaminants may be toxic to fish. In an interesting aside, NP, which is highly toxic to aquatic life (Yoshimura 1986), did not cause toxicity at the doses required to induce significant estrogenic effects. This is similar to the findings of Lech et al. (1996) who exposed rainbow trout to NP through water and found that the EC50 of Vg induction was an order of magnitude less than the LC50. The failure of several compounds, identified in both mammals and fish as estrogenic, to induce vitellogenesis suggests that a chronic, low exposure regime may be more effective in evoking responses to weak estrogenic compounds. A chronic regime would also avoid the acute toxicity that may act to prevent the appropriate concentration of compound to accumulate at the target tissues prior to lethality.

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## REFERENCES

Chadwick, R. W., R. L. Cooper, J. Chang, G. L. Rehnberg and W. K. McElroy. 1988. Possible antiestrogenic activity of lindane in female rats. J. Biochem. Toxicol. 3:147-158.

Denison, M. S., J. E. Chambers and J. D. Yarbrough. 1981. Persistent vitellogenin-like protein and binding of DDT in the serum of insecticide-resistant mosquitofish (*Gambusia affinis*). Comp. Biochem. Physiol. 69C:109-112.

Eroschenko, V. P. 1981. Estrogenic activity of the insecticide chlordecone in the reproductive tract of birds and mammals. J. Toxicol. Environ. Health 8:731-742.

Fritsch, M. K. 1991. Estrogens, progestins, and oral contraceptives. In L.B. Wingar, Jr., T.M. Brody, J. Larner and A. Schwartz, eds., Human Pharmacology: Molecular to Clinical. St. Louis: Mosby Year Book.494-514.

Goodwin, A. E., J. M. Grizzle, J. T. Bradley and B. H. Estridge. 1992. Monoclonal antibody-based immunoassay of vitellogenin in the blood of male channel catfish (*Ictalurus punctatus*). Comp. Biochem. Physiol. 101B:441-446.

Jobling, S. and J. P. Sumpter. 1993. Detergent components in sewage effluent are weakly oestrogenic to fish: An in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aq. Toxicol. 27:361-372.

Jordan, V. C. 1984. Biochemical pharmacology of antiestrogen action. Pharmacol. Rev. 36:245-276.

Kupfer, D. and W. H. Bulger. 1980. Estrogenic properties of DDT and its analogs. In J. A. McLachlan, ed., Estrogens in the Environment. Elvsevier North Holland, Inc. 239-263.

Lech, J. J., S. K. Lewis and L. Ren. 1996. *In vivo* estrogenic activity of nonylphenol in rainbow trout. Fund. Appl. Toxicol. 30:229-232.

Pelissero, C., G. Flouriot, J. L. Foucher, B. Bennetau, J. Dunogues, F. Le Gac and J. P. Sumpter. 1993. Vitellogenin synthesis in cultured hepatocytes; and in vitro test for the estrogenic potency of chemicals. J. Steroid Biochem. Molec. Biol. 44:263-272.

Raizada, R. B., P. Misra, I. Saxena, K. K. Datta and T. S. S. Dikshith. 1980. Weak estrogenic activity of lindane in rats. J. Toxicol. Environ. Health 6:483-492.

Sheahan, D. and J. Harries. 1992. Effects of Trace Organics on Fish. A joint study between the Directorate of Fisheries Research of the Ministry of Agriculture, Fisheries and Food, Brunel University and The Water Research Centre, Final Report.

Soto, A. M., H. Justicia, J. W. Wray and C. Sonnenschein. 1991. *p*-Nonyl-phenol: An estrogenic xenobiotic released from "modified" polystyrene. Environ. Health Persp. 92:167-173.

Srivastava, A. K. and A. K. Srivastava. 1994. Effects of chlordecone on the gonads of freshwater catfish, *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol. 53:186-191.

Thomas, P. and J. Smith. 1993. Binding of xenobiotics to the estrogen receptor of spotted seatrout: A screening assay for potential estrogenic effects. Marine Environ. Res. 35:147-153.

Wester, P. W. and J. H. Canton. 1986. Histopathological study of *Oryzias latipes* (medaka) after long-term β-hexachlorocyclohexane exposure. Aq. Toxicol. 9:21-45.

Wester, P. W., J. H. Canton and A. Bisschop. 1985. Histopathological study of *Poecilia reticulata* (guppy) after long-term β-hexachlorocyclohexane exposure. Aq. Toxicol. 6:271-296.

White, R., S. Jobling, S.A. Hoare, J. P. Sumpter and M. G. Parker. 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135:175-182.

Yoshimura, K. 1986. Biodegradation and fish toxicity of nonionic surfactants. JAOCS 63:1590-1596.

**Table 1. Hepatic-somatic indices (HSI) following treatment with model compounds.**

| Treatment | n | Dose (mg/kg) | Estradiol Equimolar Dose (mg/kg) | HSI % Agar Control[a] | |
|---|---|---|---|---|---|
| Agar (negative) | | -- | -- | 100.0 | (20.0) |
| E2 (positive) | 8 | 0.6 | -- | 120.0 | (20.0) |
| EE2 | 8 | 0.66 | 0.6 | 140.0[b] | (30.0) |
| ME2 | 8 | 0.68 | 0.6 | 150.0[b] | (20.0) |
| EE2 + E2 | 7 | | | 180.0[b] | (40.0) |
| ME2 + E2 | 7 | | | 190.0[b] | (20.0) |
| DES | 8 | 0.59 | 0.6 | 92.3 | (15.4) |
| DES + E2 | 8 | | | 130.8[c] | (15.4) |
| Low TMX | 8 | 0.82 | 0.6 | 91.7 | (16.7) |
| High TMX | 8 | 136.4 | 100 | 108.3 | (16.7) |
| Low TMX + E2 | 8 | | | 115.4 | (15.4) |
| High TMX + E2 | 8 | | | 100.0 | (15.4) |
| Low NP | 7 | 79 | 100 | 100.0 | (13.3) |
| High NP | 6 | 237 | 300 | 93.3 | (13.3) |
| Low NP + E2 | 8 | | | 113.3 | (13.3) |
| High NP + E2 | 7 | | | 113.3 | (20.0) |
| Low MXC | 8 | 127 | 100 | 100.0 | (14.3) |
| High MXC | 8 | 380 | 300 | 78.6 | (28.6) |
| Low MXC + E2 | 8 | | | 107.1 | (14.3) |
| High MXC + E2 | 5 | | | 92.9 | (21.4) |

[a]Mean values for percent of agar control (standard deviation).
[b]Values significantly different from E2 treatment ($p<0.05$, ANOVA with Tukey's post hoc test).
[c]Values significantly different from agar control ($p<0.05$, ANOVA with Tukey's post hoc test).

**Table 2. Mammalian xenoestrogens which did not induce vitellogenesis.**

| Chemical | Highest Dose mg/kg | Equimolar Estradiol Dose mg/kg |
|---|---|---|
| *o,p'*-DDT | 390 | 300 |
| Chlordecone | 90 | 100 |
| β-HCH | 320 | 300 |
| Lindane | 107 | 100 |

**Table 3. Vitellogenin (Vg) induction of *p*-nonylphenol (NP) and methoxychlor (MXC).**

| Treatment | n | Dose (mg/kg) | Estradiol Equimolar Dose (mg/kg) | Vg (mg/ml) | |
|---|---|---|---|---|---|
| Agar | 7 | -- | -- | 0.3[a] | (0.4) |
| Low NP | 7 | 79 | 100 | 3.6 | (3.4) |
| High NP | 6 | 237 | 300 | 9.5[b] | (5.7) |
| E2 | 7 | 0.6 | -- | 355.5 | (139.4) |
| | | | | | |
| Agar | 8 | -- | -- | 0.1 | (0.1) |
| Low MXC | 8 | 127 | 100 | 2.5 | (3.3) |
| High MXC | 8 | 380 | 300 | 3.3 | (5.1) |
| E2 | 7 | 0.6 | -- | 370.0 | (98.6) |

[a]Values are means (standard deviation).
[b]Values significantly different from agar control ($p<0.05$, ANOVA, Tukey's post hoc test).

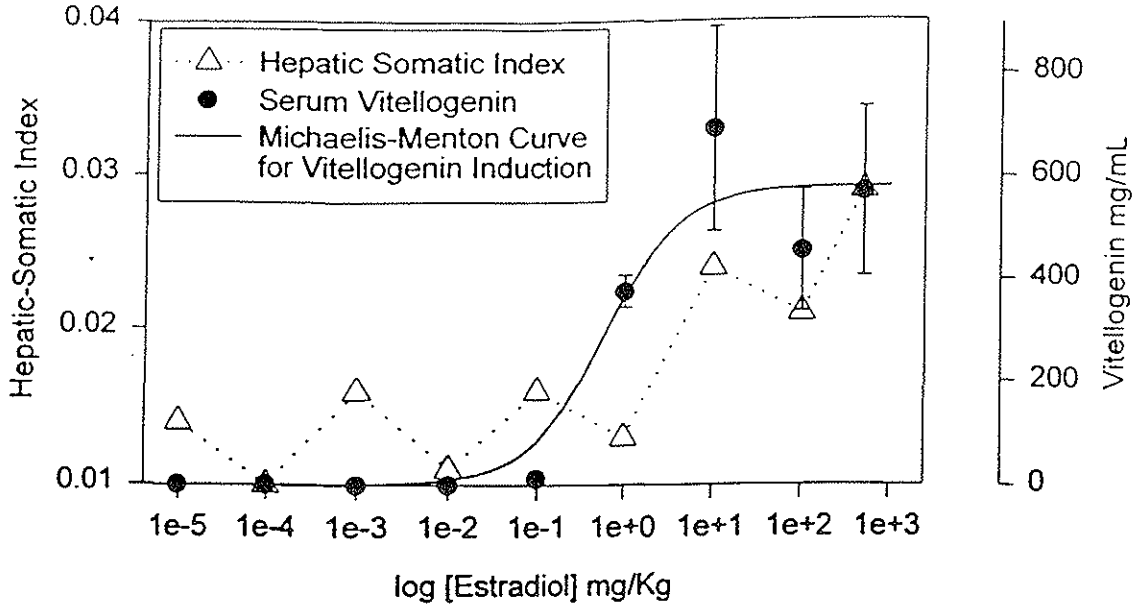


Figure 1 Dose-response of hepatic-somatic index and vitellogenin induction for estradiol. Solid line represents a Michaelis-Menton fit to vitellogenin data while error bars for vitellogenin data represent standard deviation of mean values.

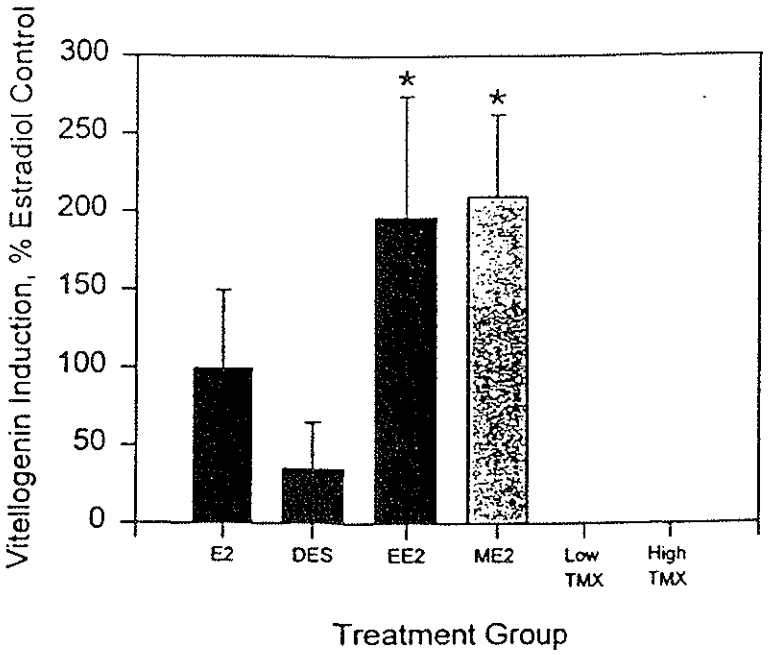


Figure 2 Vitellogenin induction following treatment with model compounds. Abbreviations: E2-estradiol, DES-diethylstilbestrol, EE2-ethinylestradiol, ME2-mestranol and TMX-tamoxifen. Low and high doses of TMX are equimolar to 0.6 and 100 mg E2/kg. * indicates statistically significant difference from E2, $p<0.05$. # indicates statistically significant difference from agar negative control, $p<0.05$.

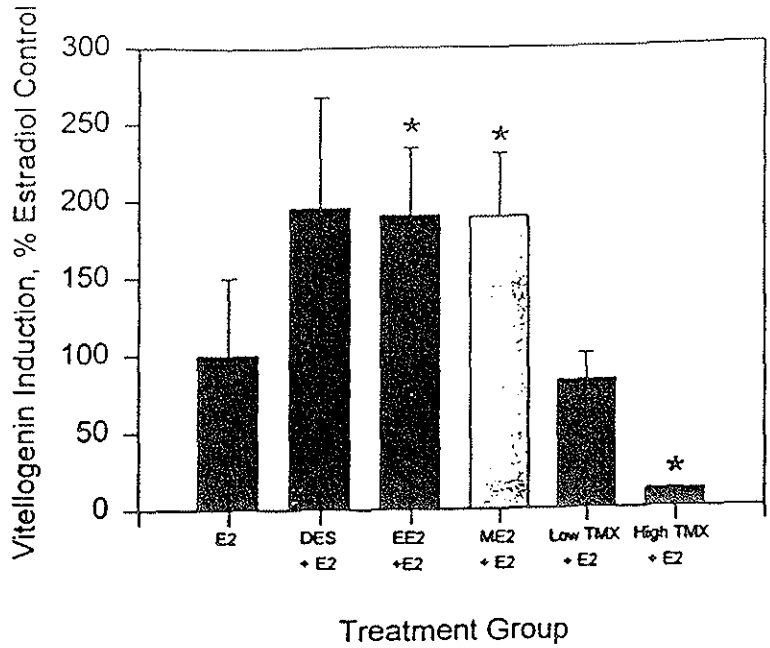


Figure 3 Vitellogenin response following treatment with combination doses (compound + E2). Abbreviations: E2-estradiol, DES-diethylstilbestrol, EE2-ethinylestradiol, ME2-mestranol and TMX-tamoxifen. Low and high doses of TMX are equimolar to 0.6 and 100 mg E2/kg. * indicates statistically significant difference from E2 alone, $p<0.05$.

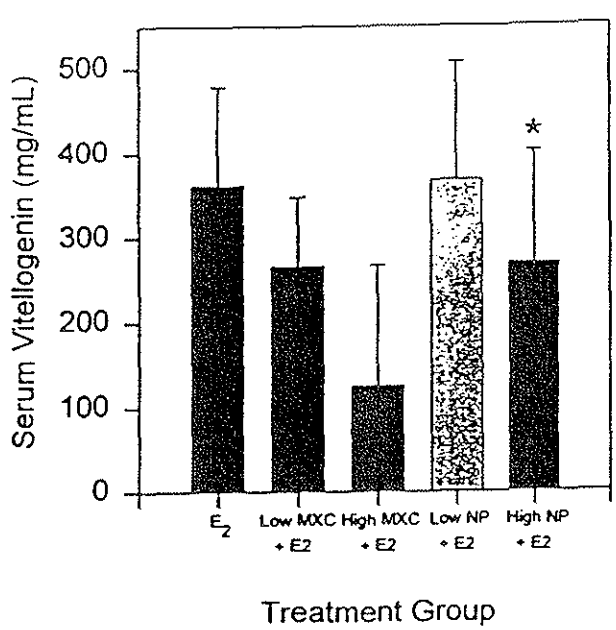


Figure 4 Vitellogenin response following treatment with combination doses of methoxychlor (MXC) or *p*-nonylphenol (NP) and estradiol (E2). Low and high doses of xenobiotic are equimolar to 100 and 300 mg E2/kg, respectively. * indicates statistically significant difference from E2 alone, $p<0.05$.