

Environmentally Persistent Alkylphenolic Compounds Are Estrogenic*

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ABSTRACT

We show that a number of alkylphenolic compounds, used in a variety of commercial products and found in river water, are estrogenic in fish, birds, and mammals. 4-Octylphenol (OP), 4-nonylphenol, 4-nonylphenoxycarboxylic acid, and 4-nonylphenoldiethoxylate were each capable of stimulating vitellogenin gene expression in trout hepatocytes, gene transcription in transfected cells, and the growth of breast cancer cell lines. The most potent of the chemicals is OP, which was able to stimulate these biological responses to a similar extent as 17 β -estradiol itself, albeit at a 1000-fold greater concentration. The action of alkylphenols is mediated by the estrogen receptor, as their effects depended on its presence and was blocked by estrogen antago-

nists. OP, 4-nonylphenol, and 4-nonylphenoxycarboxylic acid appear to possess intrinsic estrogenic activity, because they compete for binding to the estrogen receptor. Moreover, it is likely that they interact with a similar region of the hormone-binding domain as 17 β -estradiol, because the mutant receptor G-525R, which is defective in estrogen binding, is also insensitive to OP. Like 17 β -estradiol, OP is capable of stimulating the activity of both transcriptional activation functions, TAF-1 and TAF-2, in the receptor, as judged by analyzing the activity of the wild-type and mutant receptors in transiently transfected cells. The significance of our results will depend to a large extent on the degree of exposure of wildlife and humans to these estrogenic alkylphenolic compounds. (*Endocrinology* 135: 175–182, 1994)

IT IS BECOMING evident that many chemicals, both natural and synthetic, exhibit estrogenic activity (1–4). These include phytoestrogens and certain mycoestrogens, a number of pesticides and herbicides (e.g. *o,p'*-dichloro-diphenyl-trichloro-ethane and methoxychlor), some polychlorinated biphenyls, some polycyclic aromatic hydrocarbons and polychlorinated dibenzodioxins, and some alkylphenolic compounds (e.g. nonylphenol). In view of the persistence of these chemicals and their degradation products, which may also be estrogenic, and because they can be transported by water and air, such chemicals are found in all types of environment throughout the world. The majority of these chemicals are quite different in structure from the natural estrogens, so it is not possible presently to assess whether a compound is likely to be estrogenic based on a knowledge of its chemical structure. In view of this, the ability of a substance to act as an estrogen is usually discovered accidentally, as illustrated by the recent realization that the alkylphenol nonylphenol (5) and bisphenol-A (6) released from laboratory plasticware were estrogenic.

Natural estrogens (particularly 17 β -estradiol) play pivotal roles not only in controlling reproduction in females and, to a lesser extent, in males but are implicated in the development and growth of some forms of cancer (7). This accounts for the growing concern that exposure to estrogenic chemicals might cause deleterious physiological effects to both wildlife

and humans (8). In some cases, where exposure to environmental estrogenic chemicals has been considerable, effects such as precocious sexual development have been documented (9). However, recent concern has focused not so much on incidences of considerable exposure, but, rather, on whether unavoidable chronic exposure to lower concentrations of an array of estrogenic chemicals might produce less immediately obvious, but nevertheless important, effects. For example, it has been hypothesized recently that exposure to estrogenic substances might account for the increasing frequency of infertility and associated disorders of the male reproductive system in humans (10).

We have established recently that effluent from sewage treatment works contains a substance(s) that is estrogenic to fish placed in the effluent (11). We initially thought that ethinyl estradiol, originating primarily from use of the contraceptive pill, was the chemical most likely to be responsible. However, the recent report by Soto *et al.* (5) that nonylphenol was estrogenic led us to consider whether alkylphenolic chemicals, originating primarily from the use of detergents, might be responsible instead. Alkylphenol polyethoxylates (APEOs), which were introduced in the 1940s, are the second largest group of nonionic surfactants in commercial production. They are widely used, not only in detergents, but also in paints, herbicides, pesticides, and many other formulated products. APEOs with 8–12 ethoxylate groups are commonly used, nonylphenol polyethoxylates account for about 80% of APEOs (>300,000 tons are produced annually worldwide), and octylphenol polyethoxylates make up most of the remaining 20%. It has been estimated that 60% of APEOs ends up in the aquatic environment (12), most entering via sewage treatment works, where they are readily degraded to

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form relatively stable metabolites (13, 14). Some of these metabolites are hydrophobic (e.g. the alkylphenols, nonylphenol and octylphenol) and tend to accumulate in sewage sludge and river sediment, whereas others, such as short chain APEOs and carboxylic acid derivatives (APECs), are more soluble. It is, therefore, disturbing that both APEOs and APECs have been detected in drinking water in New Jersey (15).

To determine which alkylphenolic chemicals are estrogenic and to provide an estimate of their potency, we tested representatives of the most environmentally important groups of alkylphenolic compounds in assay systems spanning the vertebrates. In addition, by using mutant estrogen receptors, we assessed the extent to which the binding of alkylphenols mimics the effect of estrogen binding.

Materials and Methods

Chemicals

4-Nonylphenol (NP) was purchased from MTM (Lancashire, United Kingdom), 4-*tert*-octylphenol (OP) and 4-nonylphenoxycarboxylic acid (NP1EC) were purchased from Aldrich (Dorset, United Kingdom), and 4-nonylphenoldiethoxylate (NP2EO) was a gift from ICI (Cleveland, United Kingdom). Figure 1 shows the basic structures of these compounds. A range of octylphenol polyethoxylates, sold under the trade name Igepal, were purchased from Aldrich. These were OP2EO, OP3EO, OP5EO, and OP12EO. OP4.5EO (trade name Synperonic OP4.5) was a gift from ICI Surfactants (Cleveland, United Kingdom); this contains a mixture of ethoxylates, with a mean chain length of 4.5 ethoxylates. The estrogen antagonist 4-hydroxytamoxifen and ICI 182,780 were gifts from Dr. A. Wakeling (Zeneca Pharmaceuticals, Alderley Edge, United Kingdom). Most of these chemicals are hydrophobic, and so all were dissolved in ethanol before being tested in the various assays systems.

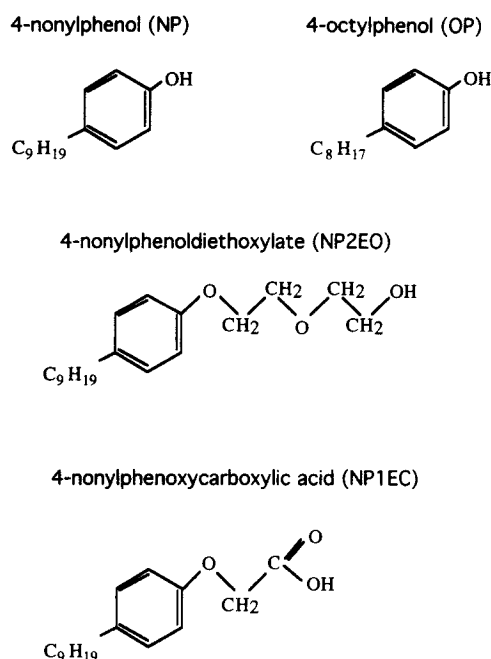


FIG. 1. Structure of the alkylphenolic compounds used in this study. The radical group (C₉H₁₉ in the case of nonylphenol, for example) is usually in a tertiary, rather than linear, confirmation.

Culture of hepatocytes from rainbow trout

The secretion of vitellogenin from primary cultures of hepatocytes has been proposed as an assay to assess the estrogenic potency of chemicals (16). Male trout served as a source of hepatocytes, because the vitellogenin gene is normally silent in males (17). Liver was perfused with a basic salt solution (pH 7.8) for 20 min to facilitate blood removal and cleavage of hepatic desmosomes. This was followed by perfusion with a salt solution containing collagenase. The liver was then removed from the body cavity and torn into small pieces to aid dispersion of the hepatocytes, which were then filtered and cultured as aggregates at a density of 1 million cells/ml in a buffered solution of phenol red-free Dulbecco's Modified Eagle's Medium (DMEM) supplemented with the nutrient mixture Ham's F-12 and 2% (vol/vol) steroid-free serum. Full details of the culture conditions can be found in the report by Jobling and Sumpter (18). Under these conditions, 17 β -estradiol stimulates stable and long term transcription of the vitellogenin gene, leading to synthesis of vitellogenin and its secretion into the medium (16, 17). Partial renewal of the medium took place every 48 h, and after the formation of aggregates, the hepatocytes were stimulated with a range of concentrations of each of the alkylphenolic compounds at each change of the medium. The concentration of vitellogenin in the medium was determined using a specific RIA (19, 20).

Cell culture

Human breast cancer cells, MCF-7 and ZR-75-1, were maintained in DMEM supplemented with 10% (vol/vol) fetal calf serum (Gibco, Paisley, Scotland, UK) and, in the case of ZR-75 cells, 10 nM 17 β -estradiol. For growth curves, cells were initially grown in phenol red-free DMEM supplemented with 10% charcoal-stripped fetal calf serum containing no hormone for 7 days. They were then transferred into medium containing 10 nM 17 β -estradiol or alkylphenols at the concentrations indicated. Chicken embryo fibroblasts (CEFs) were grown in the same medium supplemented with 1% chick serum.

DNA clones

The reporter gene pEREBCAT (21) contains an estrogen response element (ERE) derived from the vitellogenin A2 promoter up-stream of the herpes simplex viral thymidine kinase gene promoter linked to the reporter gene chloramphenicol acetyl transferase (CAT). The following mouse estrogen receptor expression vectors were used: pJ3MOR, which contains the wild-type receptor sequence (22); pJ3MOR 121-599, which contains residues 121-599 (21); and pJ3G-525R, in which the glycine at position 525 in the wild-type receptor has been replaced with an arginine residue (23).

Transfection experiments

MCF-7 and CEF cells were plated at 2×10^5 cells/6-cm dish in phenol red-free DMEM and 10% charcoal-stripped fetal calf serum and transfected using the calcium phosphate coprecipitation method, as previously described (23). The transfected DNA included a reporter plasmid pEREBCAT (5 μ g), an internal control plasmid, pJ3 luciferase (1 μ g), a mouse estrogen receptor expression vector (0.5 μ g) for experiments with CEF cells, and pJ3 Φ (24) to a total of 10 μ g/dish. After transfection, cells were maintained with no hormone, 10 nM 17 β -estradiol, alkylphenolic compounds at the concentrations indicated, 100 nM 4-hydroxytamoxifen, or 100 nM ICI 182780. After 48 h, the cells were harvested, and extracts were assayed for luciferase (25) and CAT (26) activities. Luciferase activity was used to correct for differences in transfection efficiency in all experiments. All experiments were carried out in duplicate and repeated at least twice. Mean values are presented.

Receptor binding studies

These studies were carried out using a cytosolic extract from the liver of rainbow trout because it is well documented that estradiol receptor-binding sites are present here in both male and female fish (27). Livers were removed from rainbow trout, frozen immediately in liquid nitrogen,

and subsequently stored at -80°C until required. They were then thawed and homogenized on ice in 2.5 vol buffer (50 mM Tris-HCl, 0.1 mM EDTA, 10 mM sodium molybdate, and 1 mM monothioglycerol, pH 7.4). The homogenate was centrifuged at $10,000 \times g$ for 30 min at 2°C to yield a crude nuclear pellet and a crude cytosolic supernatant. The cytosol was incubated on ice for 30 min in the presence of dextran-coated charcoal to remove any endogenous steroids, and then spun at $50,000 \times g$ for 1 h at 2°C . The final supernatant was carefully aspirated and decanted, and a saturation analysis was carried out on this cytosolic extract to establish the concentration of 17β -[2,3,7- ^3H]estradiol (86 Ci/mmol) that saturated the receptor preparation (generally between 5–10 nM). Thereafter, cytosol samples with a protein content of 2–5 mg/ml were incubated in triplicate with a saturating concentration of 5 nM tritiated estradiol, both alone and in the presence of competing ligands at a wide range of concentrations (up to 1 μM). The unbound fraction was removed by the addition of charcoal, and specific binding was quantified (27).

Results

Alkylphenolic compounds stimulate vitellogenin gene expression in trout hepatocytes

The alkylphenolic compounds OP, NP, NP2EO, and NP1EC were first analyzed for their ability to stimulate vitellogenin gene expression in trout hepatocytes. The results were compared to the effects of 17β -estradiol at 10 nM. Vitellogenin secretion was increased by all four compounds in a dose-dependent manner (Fig. 2). OP was considerably more potent than any of the other three compounds; an effect was detectable at 10^{-7} M. NP, NP2EO, and NP1EC also stimulated vitellogenin synthesis and secretion at concentrations of 10^{-6} M and above. At 10^{-5} M OP, the amount of vitellogenin secreted was almost equal to that secreted by cells exposed to 10^{-8} M 17β -estradiol.

Alkylphenolic compounds stimulate breast cancer cell growth

We then tested the mitogenic effects of the four alkylphenolic compounds on two estrogen-responsive human breast cancer cell lines, MCF-7 and ZR-75. The cell lines were initially grown in charcoal-stripped serum for 7 days

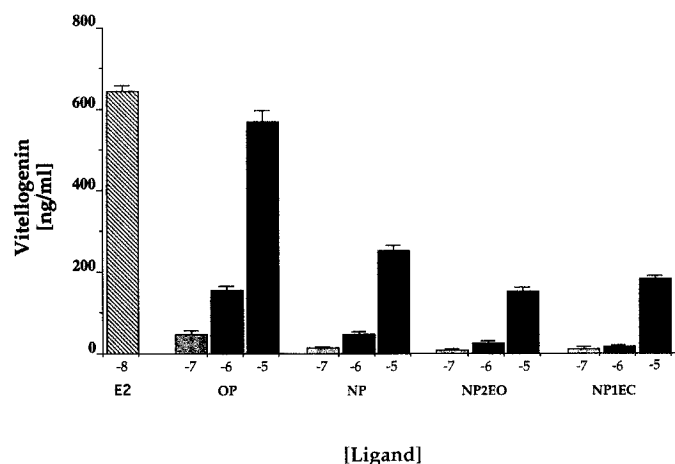


FIG. 2. Vitellogenin production from cultured hepatocytes of rainbow trout in response to alkylphenolic compounds and 17β -estradiol (E2). The compounds were tested at concentrations of 10^{-8} M (–8), 10^{-7} M (–7), 10^{-6} M (–6), and 10^{-5} M (–5).

and then in different concentrations of the compounds or 17β -estradiol for 5 days. As expected, 17β -estradiol stimulated MCF-7 cell growth, with a half-maximal effect at a concentration of 10^{-10} – 10^{-9} M. OP was the most stimulatory alkylphenolic compound; an effect was detectable at 10^{-6} M on both MCF-7 cells (Fig. 3) and ZR-75 cells (data not shown). NPIEC also stimulated cell growth, whereas NP and NP2EO were the least estrogenic. A time course for the effect of 10^{-6} M of each alkylphenolic compound indicated that OP and NPIEC stimulated the rate of growth and not just the saturation density (Fig. 4).

Alkylphenolic compounds stimulate the transcriptional activity of the estrogen receptor

To test whether alkylphenolic compounds stimulate the transcriptional activity of the estrogen receptor directly, we examined their effects in transiently transfected cells using the reporter gene EREBLCAT. Initially, we used MCF-7 cells containing endogenous receptors, then CEFs cotransfected with the mouse estrogen receptor. In MCF-7 cells, we found that OP stimulated transcription of the reporter gene EREBLCAT approximately 3-fold at a concentration of 10^{-7} M and 5- to 7-fold at 10^{-6} – 10^{-5} M, an increase similar to that obtained in the presence of 17β -estradiol (Fig. 5A). NPIEC and, to a slightly lesser extent, NP and NP2EO also stimulated transcription at concentrations in the range of 10^{-6} – 10^{-5} M.

We next investigated the effect of estrogen antagonists on the ability of 17β -estradiol and 10^{-6} M OP to stimulate EREBLCAT transcription. We found that 10^{-7} M 4-hydroxytamoxifen reduced the stimulatory effect of both 17β -estradiol and octylphenol to less than 2-fold, and the pure estrogen antagonist ICI 182780 was completely inhibitory (Fig. 5B). Thus, it appears that the effect of octylphenol was mediated by the estrogen receptor.

To confirm that the effects of the alkylphenols were mediated by the estrogen receptor, we also analyzed CEFs lacking or expressing the receptor. OP, at concentrations ranging from 10^{-7} – 10^{-5} M, also stimulated EREBLCAT transcription in CEFs providing they were cotransfected with the mouse estrogen receptor (Fig. 6). The other alkylphenolic compounds (NP, NP2EO, and NP1EC) were also active, but less potent. The observation that the transcriptional stimulation was dependent on the presence of cotransfected receptor confirms that the action of the alkylphenolic compounds was mediated by the estrogen receptor.

We next tested a series of octylphenol ethoxylate compounds, whose side-chains ranged from 2–12 ethoxylates, for their ability to stimulate EREBLCAT transcription at 10^{-6} M in MCF-7 cells. It can be seen in Fig. 7 that OP lacking an ethoxylate side-chain was the most estrogenic. The presence of two ethoxylates markedly reduced estrogenic activity, and compounds with side-chains of three or more had a negligible effect on transcription.

Alkylphenol binding mimics that of 17β -estradiol to the estrogen receptor

The estrogen receptor stimulates transcription by means of two activation regions, TAF-1 in the N-terminal domain

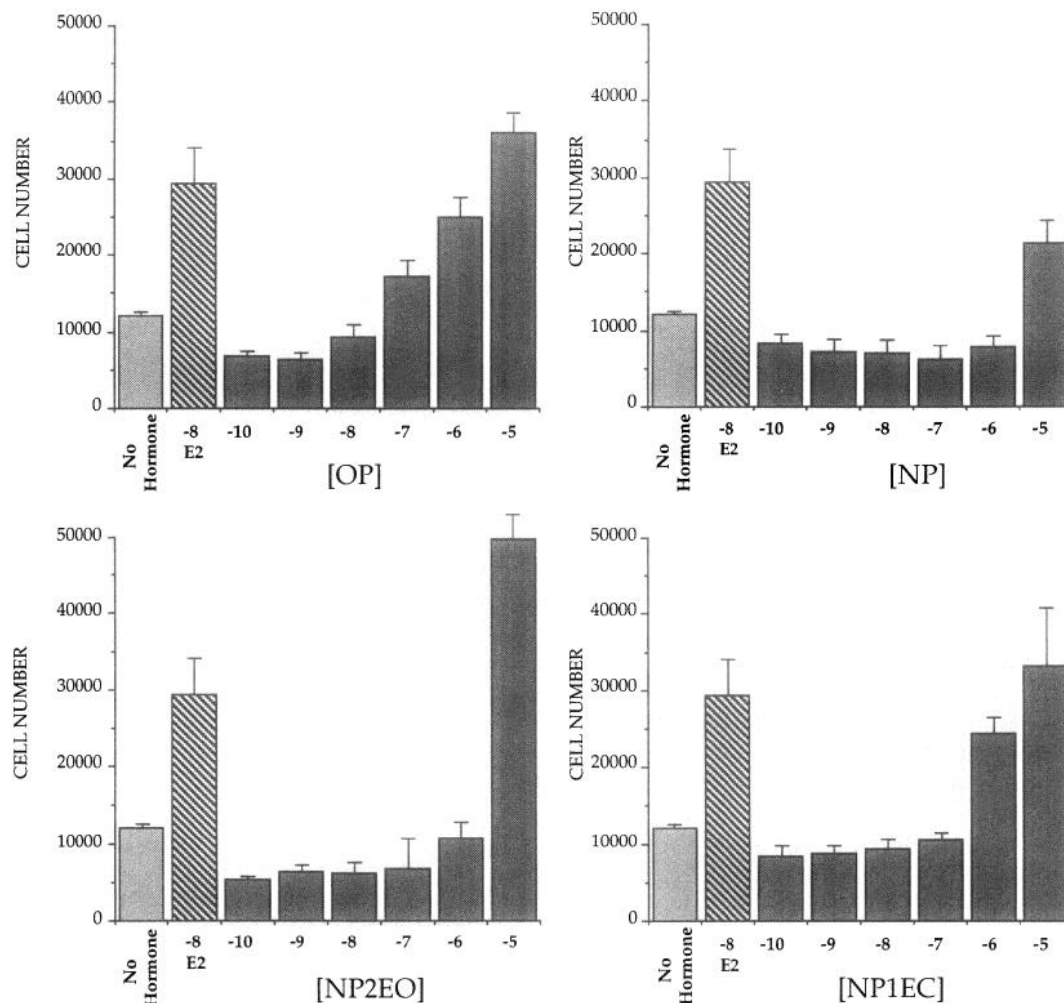


FIG. 3. Effect of alkylphenolic compounds on the growth of MCF-7 human breast cancer cells. The compounds were tested at concentrations ranging from 10^{-10} M (-10) to 10^{-5} M (-5) for 5 days. Values presented are means of at least four independent observations. E₂, 17 β -Estradiol.

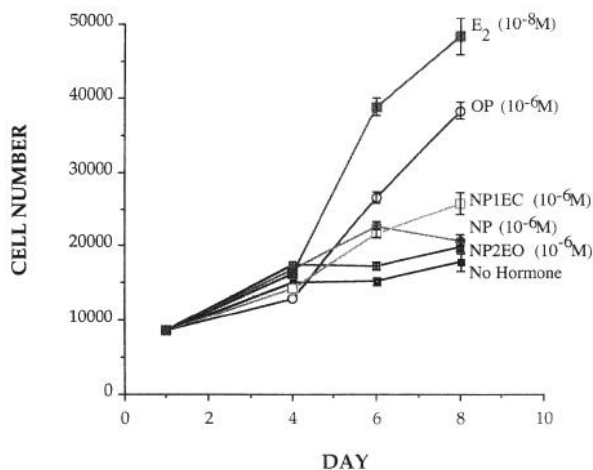


FIG. 4. Time course of the effects of alkylphenolic compounds on the growth of ZR-75 human breast cancer cells. The curves shown are representative of three independent experiments, which gave similar results. E₂, 17 β -Estradiol.

and TAF-2 in the hormone-binding domain (21, 28, 29). We investigated whether both activation regions were functional in the presence of OP by comparing the transcriptional activity of the deletion mutant MOR 121-599, lacking TAF-1, with that of the wild-type receptor MOR using the reporter EREBLCAT. As the basal transcriptional activity of each receptor varies, we have presented their activities relative to that of the reporter alone (Fig. 8). Thus, the wild-type receptor MOR, in the absence of added ligand, stimulates transcription approximately 14-fold relative to the reporter alone. This is increased a further 8-fold by 17 β -estradiol and 6-fold by OP, similar to that observed in Fig. 6. The total overall induction achieved with MOR 121-599 was reduced from approximately 100-fold to 25- to 30-fold regardless of whether OP or 17 β -estradiol was tested, reflecting the deletion of TAF-1. Interestingly, the transcriptional activity of MOR 121-599 is stimulated by 17 β -estradiol and OP to the same extent as the wild-type receptor, namely 8- and 6-fold. As this transcriptional activity was inhibited by tamoxifen, it seems likely that it was mediated by TAF-2. Thus, OP binding appears to mimic 17 β -estradiol binding in stimulat-

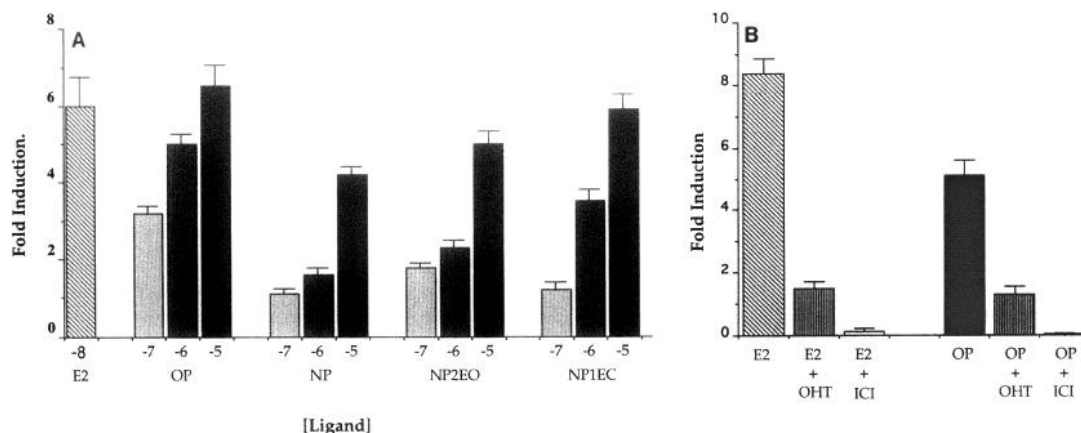


FIG. 5. Effects of alkylphenolic compounds and estrogen antagonists on transcriptional activity in MCF-7 human breast cancer cells. A, The transcriptional activity of the reporter gene EREBLCAT was determined in the presence of the compounds at concentrations ranging from 10^{-7} M (-7) to 10^{-5} M (-5). B, The ability of antiestrogens to inhibit the effect of octylphenol was compared with their ability to inhibit estrogen action. Transfected cells, incubated in the presence of either 17β -estradiol at 10^{-8} M (E2) or octylphenol at 10^{-6} M (OP), were treated with 4-hydroxytamoxifen (OHT) and ICI 182,780 (ICI) at a concentration of 10^{-7} M. The fold induction represents the increase in reporter activity over that obtained in the absence of hormone and represents the mean of two transfection experiments, each carried out in duplicate.

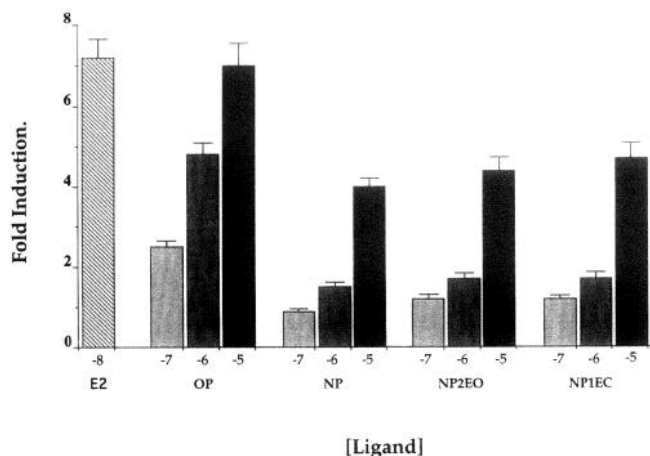


FIG. 6. Effects of alkylphenolic compounds on transcriptional activity in CEFs. The ability of the transfected estrogen receptor to stimulate the transcriptional activity of the reporter gene EREBLCAT was determined in transiently transfected CEF cells. Transcriptional activation is expressed as the fold induction of the reporter in the absence and presence of the compounds. E2, 17β -Estradiol.

ing estrogen receptor transcription in this system.

We then tested the transcriptional activity of G-525R, a mutant estrogen receptor that is incapable of binding 17β -estradiol but retains its sensitivity to 4-hydroxytamoxifen (30). OP, like 17β -estradiol, had no effect on the transcriptional activity of G-525R (Fig. 8).

Alkylphenolic compounds bind directly to the estradiol receptor

We tested the ability of the alkylphenolic compounds to compete with ^3H -labeled 17β -estradiol for binding to the trout estradiol receptor (Fig. 9). ^3H -Labeled 17β -estradiol binds to the rainbow trout estradiol receptor with a high affinity and low capacity. OP, NP, and NP1EC displaced 17β -estradiol from its receptor in a competitive manner. OP

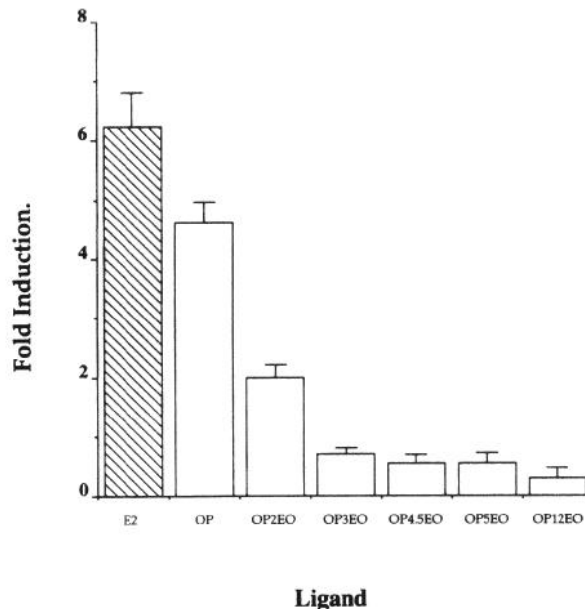


FIG. 7. The effect of increasing ethoxylate chain length on the estrogenic activity of octylphenoethoxylates. The octylphenoethoxylates at a concentration of 10^{-6} M were tested for their ability to stimulate transcription from the reporter gene EREBLCAT in transfected MCF-7 human breast cancer cells. E2, 17β -Estradiol.

was the most potent, with an approximate K_d of 1.1×10^{-5} M, whereas values calculated for NP and NP1EC were 5×10^{-5} and 2×10^{-4} M, respectively. NP2EO did not impair binding of ^3H -labeled 17β -estradiol to the estradiol receptor. Similar results were obtained for the mouse estrogen receptor (data not shown).

Discussion

Although Mueller and Kim demonstrated in 1978 that various simple alkylphenols could bind to the estradiol recep-

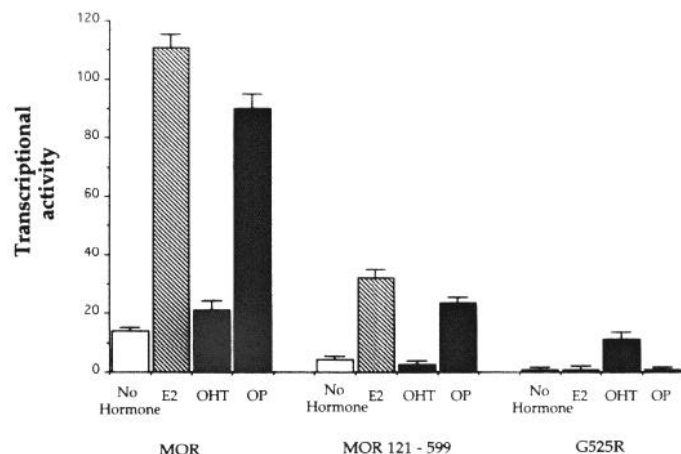


FIG. 8. The use of mutant estrogen receptors to compare the mechanism of action of octylphenol with that of 17β -estradiol. The transcriptional activity of EREBLCAT was determined in CEF cells transfected with the wild-type receptor MOR, the N-terminal deletion mutant MOR 121-599, and the point mutant G-525R. Cells were treated with 10^{-8} M 17β -estradiol (E2), 10^{-7} M 4-hydroxytamoxifen (OHT), or 10^{-6} M octylphenol (OP). The transcriptional activity represents reporter activity in the presence of receptor and ligand compared with that of reporter alone.

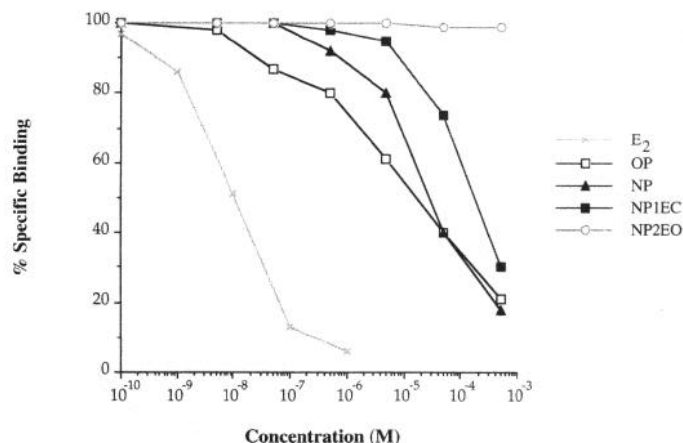


FIG. 9. Competitive displacement of tritiated 17β -estradiol (E₂) from the estrogen receptor of the rainbow trout by alkylphenolic compounds.

tor (31), their work appears to have gone largely unnoticed and resurfaced only recently when Soto *et al.* (5), after an intriguing piece of detective work, discovered that *p*-nonylphenol (4-nonylphenol) was leaching from their laboratory plasticware and acting as a weak estrogen. It was subsequently shown that a range of alkylphenols with an alkyl group in the *p* (or fourth) position on the phenol ring were able to stimulate the growth of MCF-7 cells (32). As we had established that effluent from sewage treatment works was estrogenic to fish (11) (see introduction), we tested the effect of a number of alkylphenolic compounds and confirmed that they were estrogenic to fish (18). In the present study we have focused on the environmentally important alkylphenolic compounds and extended our studies to higher vertebrates by using avian and mammalian cells, and we have begun to investigate the mechanisms by which alkylphenolic compounds act as estrogens.

The majority of APEOs enter the aquatic environment via sewage treatment works, with concentrations in the low micromolar range (~ 1 mg/liter) in the influent and 10-fold less in the effluent, although concentrations of about 1 mg/liter have been recorded in effluent from textile works and pulp mills (33). The major degradation products of APEOs are NP and OP, but because they are hydrophobic, they are found primarily in the sludge of sewage treatment works and the sediment of rivers, with concentrations of 4 g NP/kg sludge reported (34). These and the more soluble, short chain carboxylic acid derivatives (14) have been detected not only in river water (13), but also ground water (35) and tap water in the U.S. (15). In the most detailed study to date, Clark *et al.* (15) found over 20 closely related alkylphenolic compounds (all ethoxylate and carboxylate acid derivatives of nonyl and octylphenol) in drinking water in New Jersey. Our choice to investigate NP, OP, NP2EO, and NP1EC in this study was based on these chemicals being representatives of the major groups of alkylphenolic compounds present in the environment.

In this paper we show that these alkylphenols are estrogenic in fish, avian, and mammalian cells and that they mimic the effects of 17β -estradiol by binding to the estrogen receptor. OP was 10- to 20-fold more potent than NP and probably more potent than the other alkylphenols studied by Soto *et al.* (32). The APEOs and APECs are also estrogenic, but these, together with OP and NP, are 10^3 - 10^4 less potent than 17β -estradiol itself. In general, the order of estrogenicity was OP > NP1EC > NP = NP2EO regardless of the assay system used; the slight variations observed might be accounted for if further metabolism takes place in intact cells. Nevertheless, the effects of the alkylphenols appear to be mediated by the estrogen receptor itself, because their actions depend on the presence of estrogen receptors, and the effects of OP were inhibited by estrogen antagonists. Moreover, OP, NP, and NP1EC seem to possess intrinsic estrogenic activity, because they compete for binding to the estrogen receptor.

The estrogenicity of APEOs depends on their chain length; APEOs with more than three ethoxylates have little, if any, estrogenic activity. The difference in bioactivity is not due to differences in toxicity, because the most potent compounds, OP and NP, are also the most toxic, with toxicity decreasing as the ethoxylate chain lengthens (36). None of the compounds was toxic at the concentrations used, namely 10^{-5} M or lower. We cannot be certain that the weak estrogenic activity shown by the short chain APEOs (such as OP2EO) is due to the intrinsic activity of the ethoxylate, because they are inactive in receptor studies performed using either fish or mammalian (Jobling S, and SA Hoare, unpublished data) estrogen receptors. It is possible that the APEOs are degraded within cells to APs, which then show estrogenic activity. Unfortunately, no information is available on the metabolism of APEOs in cells. The single carboxylic acid derivative of an APEO that we tested (NP1EC) appeared in all of our assay systems to be a little more potent than either NP or NP2EO, although not as potent as OP. The carboxylic acid derivatives of octylphenol polyethoxylates should also be tested because of their reported presence in drinking water (15), but none

is available commercially at the present time.

Using four chemicals that are representative of the environmentally important alkylphenolic compounds, we obtained very similar results in all of our cell-based assay systems, whatever the origin of the cells. Thus, OP was considerably more potent than NP in trout hepatocytes, CEFs, and human breast cancer cells. This suggests that variations in the amino acid sequence of the estradiol receptors from different vertebrate classes (37–39) are likely to occur in regions that are not important for the binding of alkylphenolic compounds. Moreover, the observation that the mutant receptor G-525R, which is defective in estrogen binding (30), is also insensitive to OP suggests that the alkylphenols interact with a similar region of the hormone-binding domain as does 17β -estradiol.

In spite of the low binding activity of alkylphenols, it is striking that OP is able to stimulate a number of biological responses, such as cell growth and specific gene transcription, to the same extent as 17β -estradiol itself. This suggests that both transcriptional activation functions, TAF-1 and TAF-2, are functional when OP is bound to the receptor. This is supported by comparing the activity of the wild-type receptor with that of the deletion mutant MOR 121–599. In CEF cells, the TAF-2 activity exhibited by the deletion mutant receptor was induced as well by OP as by 17β -estradiol, and maximum transcriptional activation by the wild-type receptor, which depends on TAF-1, was also induced similarly by either ligand. It seems remarkable that a molecule as structurally different from 17β -estradiol as OP might be able to mimic the action of the natural hormone in inducing full transcriptional activity of the receptor. One possible explanation for this might be that ligand binding serves only one role, namely to cause displacement of associated heat shock proteins, so that receptor dimerization, DNA binding, and transcriptional activation follow as a natural consequence and are not directly modulated by the ligand. According to this hypothesis, hormone binding would not be required to induce the formation of TAF-2 as previously suggested (28). Interestingly, the corresponding activation region in the glucocorticoid receptor stimulates transcription *in vitro* in the absence of a hormonal ligand (40).

The significance of our results will depend to a large extent upon the degree of exposure of wildlife and humans to these estrogenic alkylphenolic compounds. In view of the growing concern about the acute toxicity to aquatic organisms of the degradation products (reviewed in Ref. 41), they are no longer used in household detergents in many European countries, but they are still used in other large industrialized countries, such as the U.S. and Japan. Moreover, they are widely used in industrial detergents and other formulated products, such as paints and herbicides/pesticides, throughout the world. It seems likely that the major route of exposure will be via water, but other routes are possible; for example, NP has been shown to leach from plastic used in food processing and wrapping (42). Presently, we know of no direct evidence that the estrogenic activity possessed by this group of chemicals has been shown to be responsible for any deleterious effects in any species. However, the widespread

use of the chemicals and the persistence of their degradation products in the environment coupled with the concern about inadvertent exposure of wildlife and humans to "estrogens" (reviewed in Ref. 8) raises considerable disquiet.

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