

Interaction of Organophosphate Pesticides and Related Compounds with the Androgen Receptor

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Identification of several environmental chemicals capable of binding to the androgen receptor (AR) and interfering with its normal function has heightened concern about adverse effects across a broad spectrum of environmental chemicals. We previously demonstrated AR antagonist activity of the organophosphate (OP) pesticide fenitrothion. In this study, we characterized AR activity of analogues of fenitrothion to probe the structural requirements for AR activity among related chemicals. AR activity was measured using HepG2 human hepatoma cells transfected with human AR plus an androgen-responsive luciferase reporter gene, MMTV-luc. AR antagonist activity decreased as alkyl chain length of the phosphoester increased, whereas electron-donating properties of phenyl substituents of the tested compounds did not influence AR activity. Oxon derivatives of fenitrothion, which are more likely to undergo hydrolytic degradation, had no detectable AR antagonist activity. Molecular modeling results suggest that hydrogen-bond energies and the maximum achievable interatomic distance between two terminal H-bond capable sites may influence both the potential to interact with the AR and the nature of the interaction (agonist vs. antagonist) within this series of chemicals. This hypothesis is supported by the results of recent AR homology modeling and crystallographic studies relative to agonist- and antagonist-bound AR complexes. The present results are placed in the context of structure–activity knowledge derived from previous modeling studies as well as studies aimed toward designing nonsteroidal antiandrogen pharmaceuticals. Present results extend understanding of the structural requirements for AR activity to a new class of nonsteroidal, environmental, OP-related chemicals. *Key words:* androgen receptor, fenitrothion, homology model, HepG2 cells, organophosphates, structure–activity relationships. *Environ Health Perspect* 111:545–552 (2003). doi:10.1289/ehp.5671 available via <http://dx.doi.org/> [Online 30 October 2002]

Steroid hormone receptors generally refer to ligand-dependent transcriptional regulators controlling the activity of specific gene networks involved in endocrine function (Ing and O'Malley 1995). Evidence is accumulating that some man-made compounds may disrupt normal endocrine function by binding to steroid hormone receptors. Xenoestrogens, for example, not only appear to modulate estrogen-responsive endocrine functions but may also stimulate the growth of estrogen-dependent tumors (Safe and Zacharewski 1997; Wolff and Toniolo 1995). In addition, there are reports of environmental contaminants capable of interfering with androgen receptor (AR) function. These include chemicals such as the herbicide linuron (Gray et al. 1999c; McIntyre et al. 2000), metabolites of the fungicides vinclozolin (Gray et al. 1999b; Kelce et al. 1994; Monosson et al. 1999; Wong et al. 1995) and procymidone (Mekenyan et al. 1997; Ostby et al. 1999; Waller et al. 1996a), the insecticide methoxychlor (Gray et al. 1999a) and its metabolite HPTE (Maness et al. 1998), and the DDT metabolite *p,p'*-DDE (Gray et al. 1999c; Kelce et al. 1995). The structural diversity of these chemicals, many

in widespread use, has heightened concern about the potential of other environmental chemicals to disrupt AR function and has led to the development of models and strategies for predicting potential AR activity from chemical structure (Mekenyan et al. 1997; Waller et al. 1996a).

Quantitative structure–activity relationship (QSAR) models and qualitative SAR approaches have had some success in identifying and depicting structural features that contribute to the ability of a chemical to interact with steroid hormones, for both the estrogen receptor (ER) (Anstead et al. 1997; Fang et al. 2001; McKinney and Waller 1994; Tong et al. 1997a, 1997b, 1998; Waller et al. 1996b; Wiese and Brooks 1994) and the AR (Loughney and Schwender 1992; Mekenyan et al. 1997; Singh et al. 2000; Tucker et al. 1988; Waller et al. 1996a). In the case of environmentally occurring chemicals, studies have revealed a common pattern of steric and electronic features involved in molecular recognition and receptor binding affinity, in spite of the molecular diversity of such data sets. Of particular interest to the present study are structural elements important in interactions

of nonsteroidal ligands with the AR. Multiple lines of evidence indicate that a substituted phenyl ring, denoted the A-ring in Figure 1, is an essential structural feature in that it acts as an anchor to the molecular recognition site of AR and ER receptors (Anstead et al. 1997; Brzozowski et al. 1997; Marhefka et al. 2001; McKinney and Waller 1994; Mekenyan et al. 1997; Pike et al. 1999; Poujol et al. 2000; Waller et al. 1996a). In addition, QSAR models have shown that increased negative charge in the vicinity of the A-ring off the C3 atoms is correlated with increased AR binding affinity (Mekenyan et al. 1997; Waller et al. 1996a). The greatest structural variations in nonsteroidal AR ligands appear in the molecular region that corresponds to the D-ring C17 β hydroxyl region (Figure 1) of the natural ligand, dihydrotestosterone (DHT). In this region, QSAR studies find an increase in AR binding affinity correlates with increased negative charge of substituents, and a decrease in AR binding affinity correlates with an increase in steric bulk (Mekenyan et al. 1997; Waller et al. 1996a).

Also pertinent to discerning the structural requirements of AR activity are the results of studies aimed at optimizing antiandrogenic function for the design of pharmaceuticals (Singh et al. 2000; Teutsch et al. 1994; Tucker et al. 1988). The essential rules for antiandrogenic function extracted from these studies are *a*) an electron-deficient aromatic ring with a strong hydrogen bond (H bond) acceptor (e.g., a nitro or cyano); and *b*) an aryl-amide linkage to a carbon hosting a strong H-bond donor group at the opposite terminus of the ligand (see, e.g., the essential structural moiety depicted in Figure 2). More

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We thank P. Foster, L. You, T. Fennell, J. Preston, C. Blackman, and L.E. Gray for review of this manuscript, B. Kuyper for editorial assistance, and S. Keenan and A. Swank for technical assistance. This manuscript has been approved by U.S. EPA but does not necessarily reflect agency views and policies.

Received 1 April 2002; accepted 20 August 2002.

recent publications have reported detailed crystal structures and homology models of AR, ER, and progesterone receptor (PR) ligand binding domains (LBD) with bound agonist or antagonist ligands. These studies have provided insight into steroid–ligand binding specificity (e.g., AR vs. PR) as well as information on specific residue interactions within the LBD for steroidal and nonsteroidal bound ligands (Brzozowski et al. 1997; Marhefka et al. 2001; Matias et al. 2000; Pike et al. 1999; Poujol et al. 2000; Tanenbaum et al. 1998). Taken together, QSAR models and AR binding studies paint a consistent picture of polar substituents at opposite termini of a nonsteroidal ligand framework forming H bonds with appropriate amino acid residues within the binding pocket of the AR.

We investigated the antiandrogenic activity of the organophosphate insecticide fenitrothion [*O,O*-dimethyl-*O*-(3-methyl-4-nitrophenyl) phosphorothionate]. Fenitrothion is currently registered by the U.S. Environmental Protection Agency (U. S. EPA) only for non-food uses (e.g., on terrestrial and greenhouse plants and in rat and roach baits), whereas in Japan there is extensive application of fenitrothion to food crops such as rice and fruit [estimated production of 1,230 tons for the year 2000, with estimated half-lives of 22 days and 84 days (at pH 7, 22°C) in soil and water, respectively, and a bioconcentration factor of 246]. Fenitrothion has apparent structural similarities with the pharmaceutical antiandrogen flutamide and the environmental antiandrogens vinclozolin and linuron (Figure 2) but differs most significantly in having a thiophosphonyl group as a proposed H-bond acceptor in the D-ring region of the AR interaction site. We have demonstrated that fenitrothion acts as an AR competitive antagonist *in vitro* and inhibits the development of androgen-dependent tissues *in vivo* (Tamura et al. 2001). In addition, we have reported that fenitrothion, similar to hydroxyflutamide, demonstrated weak agonist activity at high doses in a noncompetitive binding assay. To gain a better understanding of the structural requirements for AR activity for organophosphate-like chemicals, we investigate here the AR activity of a number of compounds structurally related to fenitrothion and to other known AR antagonists such as flutamide and linuron (Figure 2). The results

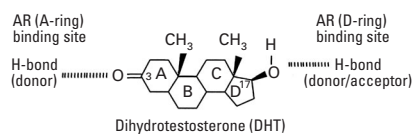


Figure 1. Schematic labeling the main ligand interaction features of the natural ligand, dihydrotestosterone, with the androgen receptor.

of this study point to common structural elements necessary for AR antagonist activity and indicate that differences in H-bond properties and the distance between two capable H-bond sites can account for differences in the nature of the AR activity within this class of antiandrogenic compounds. We consider our findings in the context of recently published crystallographic and homology models of the AR-LBD, as well as in relation to prior SAR studies and current hypotheses regarding the structural basis for antiandrogenic activity of nonsteroidal chemicals.

Materials and Methods

Chemicals. Fenitrothion, methyl parathion, and 3-methyl-4-nitrophenol were obtained from Chem Service (West Chester, PA, USA). Other organophosphorus derivatives were synthesized according to published methods (Nishizawa et al. 1961). The structure of the synthesized compounds was determined as follows: ^1H NMR spectra were obtained on a JEOL EX400 spectrometer (JEOL, Tokyo, Japan) using tetramethylsilane as an internal standard. Infrared and UV spectra were recorded on a Perkin-Elmer Spectrum RX III FT-IR (Perkin-Elmer, Beaconsfield, Bucks, UK) and Shimadzu UV-240 spectrophotometer (Shimadzu, Kyoto, Japan), respectively. Spectral data were consistent with assigned structures. Refractive index was measured using a 1T Abbe Refractometer (ATAGO Co., Tokyo, Japan). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA). All chemicals were > 97% pure.

Plating and transfection. We performed transfection experiments as previously described (Maness et al. 1998; Tamura et al.

2001). Briefly, human hepatoma HepG2 cells (ATCC, Rockville, MD, USA) were plated in triplicate in 24-well plates (Falcon Plastics, Oxnard, CA, USA) at a density of 10^5 cells/well in complete medium (phenol red-free Eagle's minimal essential medium; GIBCO/BRL, Grand Island, NY, USA) supplemented with 10% resin-stripped fetal bovine serum (Hyclone, Logan, UT, USA), 2% L-glutamine, and 0.1% sodium pyruvate and allowed to incubate overnight at 37°C in a humidified atmosphere of 5% CO_2 /air. Cells were then transfected as previously described (Maness et al. 1998) (SuperFect; Qiagen, Valencia, CA, USA, or TransIT; Mirus Co., Madison, WI, USA) with three plasmids: *a*) 10 ng/well human AR, *b*) 405 ng/well MMTV-luc reporter plasmid, and *c*) 40 ng/well pCMV β -gal plasmid as a transfection and toxicity control. Cells were placed in a 37°C incubator with a humidified atmosphere of 5% CO_2 /air for 3 hr. The transfected cells were then rinsed with phosphate-buffered saline and treated with various concentrations of test chemical from 10^{-8} to 10^{-5} M for determination of AR agonist activity.

We performed dose-shift experiments for the determination of AR antagonist potency by adding set concentrations of test chemical (10^{-7} , 10^{-6} , 10^{-5} M) across a complete dose–response range of the natural ligand dihydrotestosterone (DHT). A vehicle control was included in each experiment (dimethyl sulfoxide; Sigma) in complete medium. Final concentration of dimethyl sulfoxide in medium was 0.1%. After a 24-hr incubation, treated cells were rinsed with phosphate-buffered saline and lysed with 65 μL of lysing buffer (25 mM tris-phosphate, pH 7.8, 2 mM 1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid,

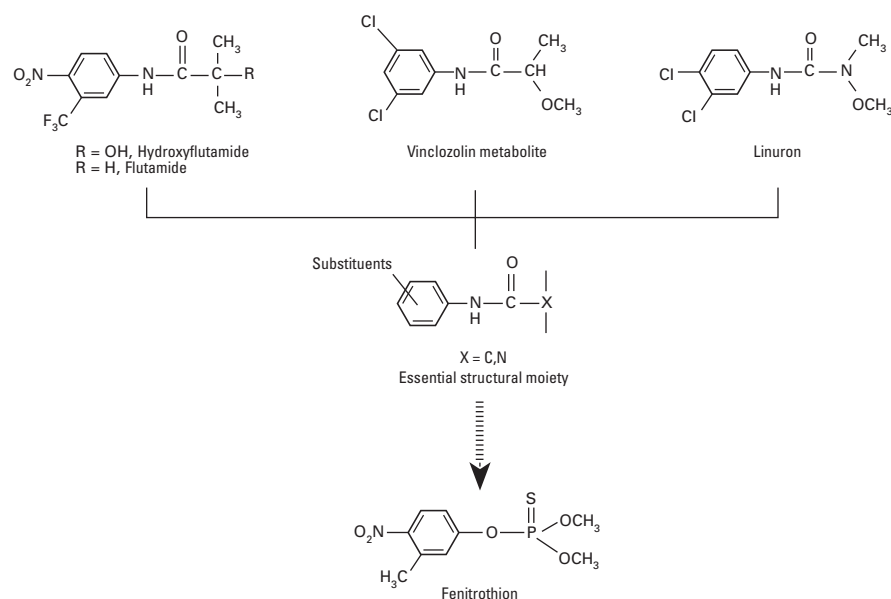


Figure 2. Structural similarity between known nonsteroidal androgen-receptor antagonists and fenitrothion.

10% glycerol, 0.5% Triton X-100, 2 mM dithiothreitol). Lysate was divided into two 96-well plates for luciferase and β -galactosidase activity determination.

Enzyme activity assay. We added luciferase assay reagent (100 μ L; Promega, Madison, WI, USA) to 20 μ L of lysate and determined luminescence immediately using an ML3000 microtiter plate luminometer (Dynatech Laboratories, Chantilly, VA, USA). For β -galactosidase activity determination, 20 μ L of a 4 mg/mL solution of chlorophenol red- β -D-galactopyranoside (CPRG; Sigma) and 150 μ L CPRG buffer (60 mM Na_2HPO_4 , 40 mM NaH_2PO_4 , 10 mM KCl, 1 mM MgSO_4 ,

50 mM β -mercaptoethanol, pH 7.8) was added to 30 μ L of lysate. Absorbance at 570 nm was determined over a 30-min period using a V_{max} kinetic microplate reader (Molecular Devices, Menlo Park, CA, USA).

Statistical analysis. Unless otherwise noted, measured activities presented in this study represent the means \pm SE resulting from at least three separate experiments with triplicate wells for each treatment dose. We analyzed dose–response data using the sigmoidal dose–response function of the graphical and statistical program Prism (GraphPad, San Diego, CA, USA). The equilibrium dissociation constant (K_B) for the

antagonist–receptor complex was determined by Schild regression analysis of the dose ratio as previously described (Maness et al. 1998; McIntyre et al. 2000; Tamura et al. 2001). The dose ratio is $[A']/[A]$, where $[A']$ and $[A]$ refer to equiactive concentrations of DHT in the presence and absence of antagonist, respectively (Kenakin 1993).

Computation of structural properties. We calculated ligand interatomic distances and H-bond energies using the SPARTAN molecular modeling software (version 5.1.1 for UNIX; Wavefunction, Inc., Irvine, CA, USA). Geometries of studied molecules were fully optimized using the semiempirical PM3 method (Stewart 1998). To sample the range of thermodynamically achievable distances between putative H-bond acceptor sites, limited conformational space was explored by constraining the two putative H-bond acceptor atoms to be a fixed distance apart, allowing the remainder of the molecule to relax to the lowest energy configuration, followed by release of the original constraint allowing full relaxation in the extended conformation. We tabulated distances between putative H-bond acceptor sites, atomic charges at these sites, and total molecular energies of the conformers. We estimated H-bond interaction energies of the putative donor and acceptor sites on the natural AR ligand, DHT, and selected nonsteroidal analogues using the PM3 method to compute the energies of interaction with a single water molecule at each site; PM3 fully optimized conformations of the water-bound species were computed for this purpose.

Table 1. Equilibrium dissociation constants (K_B) of organophosphate compounds and structurally related androgen receptor antagonists.

Chemical name ^a	No.	R ₁	R ₂	R ₃	X	K_B (x 10 ⁻⁸ M)
Fenitrothion	1	CH ₃	NO ₂	CH ₃	S	2.18 ^b
	2	CH ₃	NO ₂	CH ₃	O	ND ^c
Methylparathion	3	CH ₃	NO ₂	H	S	35.9
	4	CH ₃	NO ₂	H	O	ND
	5	C ₂ H ₅	NO ₂	CH ₃	S	16.5
Ethylparathion	6	C ₂ H ₅	NO ₂	H	S	ND
	7	C ₂ H ₅	CH ₃	H	S	ND
	8	C ₂ H ₅	H	CH ₃	S	ND
	9	C ₂ H ₅	(O-CH ₂ -O)	CH ₃	S	ND
10	<i>n</i> -C ₃ H ₇	NO ₂	CH ₃	S	ND	
Flutamide ^d						1.07 ^e
Hydroxyflutamide ^d						0.22
Linuron ^d						75.8 ^f

^aIUPAC chemical names: 1, *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothionate; 2, *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphate; 3, *O,O*-dimethyl *O*-4-nitrophenyl phosphorothionate; 4, *O,O*-dimethyl *O*-4-nitrophenyl phosphate; 5, *O,O*-diethyl *O*-(3-methyl-4-nitrophenyl) phosphorothionate; 6, *O,O*-diethyl *O*-4-nitrophenyl phosphorothionate; 7, *O,O*-diethyl *O*-4-methylphenyl phosphorothionate; 8, *O,O*-diethyl *O*-3-methylphenyl phosphorothionate; 9, *O,O*-diethyl *O*-3,4-methylenedioxyphenyl phosphorothionate; 10, *O,O*-di-*n*-propyl *O*-(3-methyl-4-nitrophenyl) phosphorothionate. ^bData from Tamura et al. (2001). ^cND = K_B was not detected at the concentration of 1×10^{-5} M. ^dRefer to Figure 2 for chemical structures. ^eData from Maness et al. (1998). ^fData from McIntyre et al. (2000).

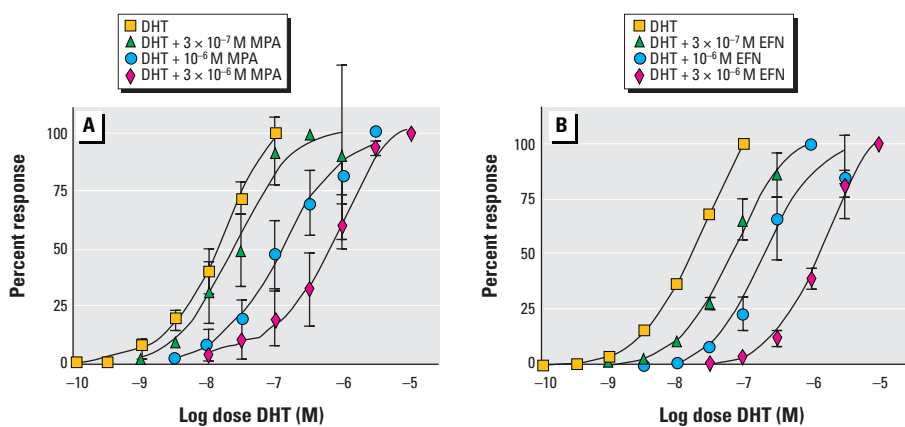


Figure 3. Methylparathion and ethylfenitrothion act as competitive reversible inhibitors of AR. HepG2 cells were transfected with human AR plus an AR-responsive luciferase reporter gene and a constitutively active β -galactosidase reporter gene (transfection and toxicity control). Transfected cells were treated with 10^{-10} to 10^{-5} M DHT either alone or in combination with 3×10^{-7} M, 10^{-6} M, and 3×10^{-6} M methylparathion (A) or ethylfenitrothion (B). Values represent the means \pm SE of three separate experiments and are presented as percent response, with 100% activity defined as the activity achieved with 10^{-7} M DHT alone in each experiment.

Results

Androgen receptor antagonist activity. We examined the interaction of a select group of fenitrothion derivatives with the AR (Table 1). Mean maximal luciferase activity achieved at 10^{-7} M DHT across all experiments was $4,889 \pm 967$ with an interassay coefficient of variation (CV) of 48%. This represents a 163-fold induction over background. When experiments were normalized to percent response, with 100% being the maximal level of induction within each individual experiment, the interassay CV in the linear portion of the curve (5×10^{-7} M DHT) was 13%. At this same dose (5×10^{-7} M DHT), the within-assay (replicate wells) CV averaged 17% across all experiments.

Of the compounds tested in this study, only methylparathion 3 and ethylfenitrothion 5 demonstrated sufficient AR antagonistic activity to determine potency in dose-shift experiments (Figure 3). Methylparathion and ethylfenitrothion both caused parallel shifts in DHT dose–response curves indicating that, similar to fenitrothion, they are competitive AR antagonists. Equilibrium dissociation constants (K_B) (Kenakin 1993) for methylparathion and

ethylfenitrothion were determined as previously described (Maness et al. 1998; McIntyre et al. 2000; Tamura et al. 2001) and are presented in Table 1. Cytotoxicity was not observed for any of the tested compounds in the selected dose range (data not shown).

Methylparathion 3, which differs from fenitrothion 1 only by absence of the *m*-methyl substitution on the phenyl ring, was approximately 16-fold less potent than fenitrothion (Table 1). Similarly, ethylfenitrothion 5 demonstrated AR antagonist activity, whereas ethylparathion 6 gave no detectable antiandrogenic activity up to a maximum concentration of 10^{-5} M (Table 1). These results indicate that *m*-methyl substitution enhances AR antagonist activity of this class of compounds.

Ethylfenitrothion 5 was 8-fold less potent than fenitrothion 1 (Table 1). The *n*-propyl ester derivative 10 displayed weak AR antagonist activity; however, toxicity of this compound $> 10^{-5}$ M interfered with our ability to perform experiments necessary to determine K_B . However, the AR antagonist activity of the *n*-propyl ester derivative 10 was weaker than that observed for methylparathion 3

(Table 1). The observation that the antagonist activity decreases as alkyl chain length increases indicates that the increase in steric bulk in the vicinity of thiophosphonyl group inhibits AR binding affinity.

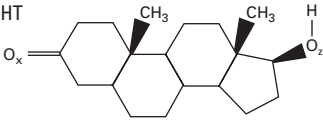
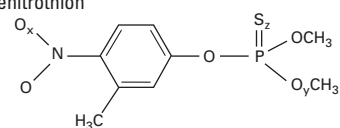
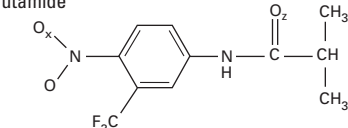
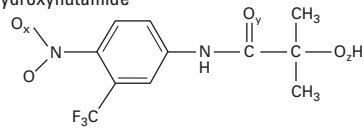
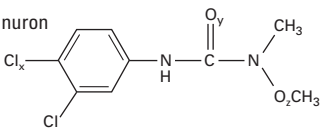
Oxon derivatives 2 and 4 (Table 1) are isoelectronic to fenitrothion 1 and methylparathion 3, respectively, and would be expected to react similarly, and yet these derivatives showed no antiandrogenic activity at the highest concentration tested (10^{-5} M). Because oxon derivatives of organophosphorus compounds are more easily degraded by hydrolysis than are thiono derivatives (Eto 1974), our results suggest that the oxon derivatives 2 and 4 are unstable under the experimental conditions used in this study.

None of the tested derivatives in which the nitro group at position R2 (Table 1) was replaced by a methyl, 7, a hydrogen, 8, or a methylenedioxy group, 9, gave detectable antiandrogenic activities up to the highest concentration tested (10^{-5} M). The most obvious explanation is the lack of hydrogen bond acceptor capability at this site in 7 and 8 and the weaker and more conformationally constrained H-bond acceptor capability of chlorine in

derivative 9. A metabolite of fenitrothion, 3-methyl-4-nitrophenol, in which the phosphothionylester group is cleaved, also showed no detectable activity (data not shown).

Androgen receptor agonist activity. In addition to their antagonist activity, hydroxyflutamide and fenitrothion have been shown to have weak agonist activity at high concentrations in the absence of effective competition of the natural ligand (Maness et al. 1998; Tamura et al. 2001). To explore the structural basis for this AR agonist activity, we computed steric and electronic properties of putative H-bond sites for DHT and for four nonsteroidal compounds with AR agonist and antagonist activity (Table 2). For each molecule listed in Table 2, the first row of numbers corresponds to properties computed for the calculated equilibrium geometries (denoted $E = 0.0$ kcal/mol). In addition, the first two columns of data indicate the relative energy cost (from equilibrium) required to achieve a fixed nonequilibrium distance ($x-z$) between the most distant H-bond centers. All five of these molecules have some degree of flexibility and can achieve shortening (in the case of DHT) or lengthening (in the case of the

Table 2. Calculated properties of dihydrotestosterone (DHT) in relation to fenitrothion and known androgen antagonists.

Chemical structure	Relative energy (kcal/mol) ^a	Distance (Å) ^b $x-z$	Atomic charge ^c			H bond (kcal/mol) ^d		
			x	y	z	x	y	z
DHT 	0.00 0.36	10.48 10.00	-0.31	-0.30	-3.70 (1.81 Å)	-3.14 (1.82 Å)		
Fenitrothion 	0.00 1.68	8.18 9.50 (8.09) ^e	-0.60	-0.58	-0.59	-3.33 (1.83 Å)	-0.17 (1.88 Å)	-2.17 (2.83 Å)
Flutamide 	0.00 2.48	7.50 8.00	-0.58		-0.33	-2.91 (1.85 Å)		-2.97 (1.82 Å)
Hydroxyflutamide 	0.00 1.72	8.94 9.50 (7.27) ^e	-0.58	-0.35	-0.31	-2.96 (1.83 Å)	-3.20 (1.81 Å)	-2.95 (1.83 Å)
Linuron 	0.00 1.65	9.16 9.50 (7.00) ^e	0.09	-0.34	-0.22	-2.06 (2.64 Å)	-3.25 (1.82 Å)	-2.85 (2.68 Å)

^aRelative energies of PM3 (Stewart, 1998) optimized geometries; $E = 0.00$ kcal/mol corresponds to fully optimized equilibrium configuration; whereas second energy corresponds to energy when $x-z$ distance is constrained (e.g., 10 Å for DHT) and rest of molecule is allowed to relax. ^bDistance between most separated H-bonding centers (O or S) in equilibrium or constrained configuration. ^cPM3 Mulliken atomic charges at equilibrium geometry. ^dH-bond interaction energies, $E(\text{product}) - E(\text{water}) - E(\text{ligand})$, computed using PM3 method, using fully optimized structures of water, ligand, and merged water-ligand product. ^eDistance of $x-y$ H-bonding centers corresponding to the constrained configuration with $x-z$ distance of 9.5 Å.

other four molecules) of the distance between H-bond centers with relatively little energy cost. However, all four of the nonsteroidal compounds fall short of the ≥ 10 Å separation of H-bond centers calculated for DHT. Of the four, flutamide has the shortest computed distance (8 Å) between H-bond oxygen centers, falling a full 2 Å short of the DHT template for the AR ligand binding interaction. Each of the remaining three nonsteroidal compounds can achieve a relatively stretched configuration (9.5 Å between the most distant H-bond centers) with small energy cost (< 1.8 kcal/mol), to within 0.5 Å of the lower bound for DHT. Of these three compounds, only linuron has no measurable agonist activity (McIntyre et al. 2000).

An important structural and functional distinction between fenitrothion and DHT is the thiol as a putative H-acceptor center in fenitrothion versus the C17 β hydroxyl in DHT. Estimated energies and distances associated with H bonds formed between a water molecule and possible H-bond sites are listed in the last three columns of Table 2. Comparison of the calculated H-bond energies and distances corresponding to the most distant z center for fenitrothion and hydroxyflutamide indicates a smaller H-bond energy for the sulfur (-2.17 vs. -2.95 kcal/mol), but also a 1 Å longer H-bond distance (2.83 vs. 1.83 Å). Hence, the smaller H-bond interaction energy may be offset by a more easily achieved H-bond distance for fenitrothion versus hydroxyflutamide. If the extra 1 Å H-bond distance is factored into the overall distance between H-bond centers, fenitrothion extends to the 10 Å range of the DHT template.

Discussion

The present study focused on identifying and characterizing the structural basis for the interaction of organophosphorus-related chemicals with the AR. In attempting to further elucidate the AR binding mechanism of organophosphorus-like ligands, such as fenitrothion, it is important to consider a proposed binding mechanism in the context of what is known generally about steroid hormone receptor function, as well as specifically about polar residue interactions of bound ligands in the AR-LBD.

A “mouse trap” mechanism based on crystallographic studies of ligand-binding interactions has been proposed as a model for ligand-receptor binding within the nuclear steroid hormone superfamily (Brzozowski et al. 1997; Buchanan et al. 2001; Goldstein et al. 1993; Renaud et al. 1995; Wurtz et al. 1996). Briefly, the ligand is trapped from the bulk solution at the entrance of the ligand-binding pocket and brought into and locked at the binding site by interaction with amino acid residues within the receptor through

H-bond and hydrophobic interactions. This leads to formation of an AR homodimer with two bound ligands, which induces additional conformational transitions leading to a more compact receptor structure and a transcriptionally active ligand-receptor homodimer complex. The specifics of the ligand binding interaction vary with each of the nuclear steroid hormone receptors, but in all cases the carboxy-terminal helix 12 appears to play a crucial role in determining agonist or antagonist activity. In the case of a bound agonist, helix 12 acts as a “lid” that swings around to entrap the ligand in the LBD, effecting the necessary conformational change for transcription to occur (Brzozowski et al. 1997; Buchanan et al. 2001; Matias et al. 2000). In the case of a bound antagonist, the helix 12 lid is prevented from closing and the entrance to the LBD remains open, thus preventing transcriptional activation (Brzozowski et al. 1997; Buchanan et al. 2001; Marhefka et al. 2001; Pike et al. 1999). With retinoic acid (Figure 2) and retinoid X receptors (RXR), it is believed that the carboxylate group of retinoic acid enters the ligand-binding pocket first and is drawn down the hydrophobic cleft to its anchoring site. Both the H bond at the carboxylate group site and Van der Waals interaction at the β -ionone ring site stabilize the ligand (Renaud et al. 1995; Wurtz et al. 1996). Likewise, for 17 β -estradiol (Figure 2), the 3-OH group of the A-ring and the C17 β hydroxylic oxygen of the D-ring both act as anchoring elements and H-bond donors within ER α (Brzozowski et al. 1997; Pike et al. 1999; Tanenbaum et al. 1998). For the DHT-AR interaction, the carbonyl oxygen of the A-ring acts as an H-bond acceptor, whereas the C17 β hydroxylic oxygen of the D-ring could act as either an H-bond donor or acceptor (Figure 2) (Marhefka et al. 2001; Matias et al. 2000).

Specifically in reference to the AR-LBD, it had been inferred from DNA sequence homology (Wurtz et al. 1996) and mutation studies (Doesburg et al. 1997; Gottlieb et al. 1998; Taplin et al. 1999; Wurtz et al. 1996) that the carbonyl oxygen of the A-ring and the C17 β hydroxylic oxygen of the D-ring in DHT most likely interact through H-bonds with Arg⁷⁵² in helix 5 and Thr⁸⁷⁷ in helix 11 in AR-LBD, respectively, because mutation of these residues caused a complete androgen-insensitivity syndrome (Sultan et al. 1993) and altered ligand specificity (Veldscholte et al. 1990). Recent crystallographic determination of the AR-LBD bound to a steroidal-type ligand (R1881), and a more recent homology modeling study that considered a variety of nonsteroidal AR ligands bound to the LBD, provide evidence to support and refine this view (Marhefka et al. 2001; Matias et al. 2000). Both studies reported H-bonding

interactions of the A-ring polar group of bound AR ligands with Arg⁷⁵², mediated by one or two bound water molecules. On the opposite terminus of the molecule, the crystallographic study provided evidence of H bonding between the C17 β hydroxyl group of R1881 with two polar residues: the carbonyl oxygen of Asn⁷⁰⁵ and the hydroxyl oxygen of Thr⁸⁷⁷ (Matias et al. 2000). The subsequent AR homology study (Marhefka et al. 2001) considered a broader variety of bound nonsteroidal ligands and found different possible binding modes within this C17 β -OH region, indicating the possibility of either an H-bond donor or acceptor interaction of the ligand with Thr⁸⁷⁷. Hence, we propose that the mechanism for the fenitrothion thiol to act as an H-bond acceptor in the D-ring 17 β -OH region of the AR-LBD likely involves interaction with the Thr⁸⁷⁷ residue of helix 11. We speculate further that the amide nitrogen of Asn⁷⁰⁵ could possibly act as an H-bond donor in interaction with the fenitrothion thiol. Comparison of a putative three-dimensional binding orientation of fenitrothion compared to DHT in the AR-LBD is represented schematically in Figure 4.

Regarding the mechanism of activation of the AR by agonist ligands, Thr⁸⁷⁷ is located within helix 11 in close apposition to helix 12. We speculate that by establishing favorable interactions with Thr⁸⁷⁷ (as well as other residues within this domain, specifically Asn⁷⁰⁵), the ligand effectively stabilizes this region of the AR. The result is to enhance the overall probability of helix 12 favorably orienting into an activated position. Analysis of two independently elucidated crystal structures of the AR [Protein DataBank (PDB) accession numbers 1I38 (Sack et al. 2001) and 1E3G (Matias et al. 2000)] reveals a distance of approximately 15.2 Å between the H-bonded terminal nitrogen of Arg⁷⁵² and the hydroxyl oxygen of Thr⁸⁷⁷, thus providing requisite dimensions within the AR-LBD (Figure 5). Allowing a maximum of 2.5–3.0 Å from each of these atoms to form stable H bonds suggests a separation distance of 9.2–10.2 Å between H-bonding groups present on candidate ligands, with the lesser of these values approaching the limit of stability for H bonding. It can be inferred that ligands that are unable to achieve thermodynamically stable conformations within these dimensional constraints will have reduced capacity to transcriptionally activate the AR and produce agonist effects.

The present analysis considers the conformationally stretched distance between polar H-bond capable sites of known AR antagonists to be a measure of the potential of a molecule to express additional agonist properties. We henceforth refer to this as the “near-10 Å polar interactions rule.” Why is it

important to consider potential agonist properties if these appear secondary to antagonist activity and are detected only at high doses in the absence of effective competition with natural ligand (Maness et al. 1998; Tamura et al. 2001)? How can we interpret this information mechanistically and in the context of a risk evaluation? Referring to the schematic representation of the AR-LBD in Figure 4, we consider the three main binding elements likely required for antagonist and/or agonist activity. In common with other known AR antagonists, fenitrothion and its organophosphorus-like AR antagonist analogues all contain the essential A-ring phenyl feature with a putative strong H-bond interaction site. We posit that structures that can achieve, in addition, effective hydrophobic and steric interactions in the central binding region of the LBD are necessary for AR antagonism.

Recent elucidation of the AR-LBD with bound ligands confirms a central binding region of nonpolar residues that is predicted to tolerate more bulky, hydrophobic substituents (Marhefka et al. 2001; Matias et al. 2000). However, structural constraints are clearly important for conferring sufficient and optimum binding affinity to produce antagonism. This contention is supported by the results of an earlier study aimed at designing high-affinity nonsteroidal antiandrogens with purely antiandrogenic activity for clinical application (i.e., with no agonist effects or cross hormonal interactions) (Teutsch et al. 1994). Selected members of the class of N-substituted-arylthiohydantoin, analogues of the common antiandrogen therapeutic agent nilutamide, were found to have exceptionally high relative binding affinities to the AR, similar to DHT. The structural modifications from previously known antagonists were primarily in the D-ring region, involving a thioamide replacement of an amide. These compounds did not, however, provide a good

candidate feature for D-ring H-bond interaction according to the “near-10 Å polar interactions rule” (the thiol was computed to be a 7.5 Å separation from the A-ring H-bond acceptor site), which is consistent with lack of reported agonist properties.

The importance of essential structural elements in the central binding region is also supported by the observed 15-fold increase in binding affinity for fenitrothion versus methylparathion upon A-ring substitution of a hydrogen for a methyl at the R3 A-ring position (see Table 1). Previous authors suggested that electron-withdrawal properties of the R3 phenyl substituent enhancing the H-bond acceptor capability at the R2 position are important for antagonism (Singh et al. 2000; Teutsch et al. 1994; Tucker et al. 1988). In contrast, the authors of a recent AR homology study counter that the steric/hydrophobic interaction aspects of this substituent are more important components for binding (Marhefka et al. 2001), a view more consistent with the present findings.

For the last of the three AR-LBD binding elements, multiple lines of evidence implicate a strong H-bond interaction in the vicinity of the D-ring (C17β-OH) LBD binding region as being a necessary, but not sufficient, requirement for AR agonist activity. SAR evidence gathered from previous study of an extensive series of nonsteroidal antiandrogens (Tucker et al. 1988), some with both agonist and antagonist properties, is consistent with this view. In that study, more than 70 compounds were synthesized and tested, all 3-(substituted thio)-2-hydroxypropionanilides (HPAs) containing the essential structural moiety illustrated in Figure 2 with an additional polar hydroxyl substituent on X. A key finding of that study in relation to the present work is that the electron-withdrawing capability of the substituent opposite to the hydroxyl group on X determined whether the molecule had agonist effects in

addition to antagonist effects: Compounds with CF₃ substituents were agonists/antagonists, whereas the same compounds with CH₃ substituents had no agonist properties and a 2- to 3-fold reduction in antagonism. The clear implication is that the X-OH becomes a stronger H-bond donor with the additional CF₃ substituent and therefore, is more likely to enhance the D-ring region interaction necessary to produce agonism. In comparison to our own work, the HPAs can be viewed as structurally analogous to hydroxyflutamide, with the same essential structural moiety yielding the same distance separations between H-bond capable polar groups as in hydroxyflutamide (Figure 2 and Table 2). Hence, all of the HPAs examined satisfy our proposed distance requirement for potential C17β-OH region interaction, and all are reportedly antagonists, but only those chemicals with sufficiently strong H-bond interaction potential in the D-ring region have additional agonist activity.

A recent study that considered the binding of steroidal and nonsteroidal ligands to a double mutant form of the AR sheds further light on the multiple factors controlling the potential for a ligand to exhibit AR agonism (Matias et al. 2002). Mutations of Thr⁸⁷⁷ (Ala) and Leu⁷⁰¹ (His) in the D-ring region were shown to inhibit binding of the natural ligand, DHT, and to significantly enhance binding and agonist properties of steroidal cortisols, as well as hydroxyflutamide. Crystallographic evidence, as well as energy minimization calculations, indicates that the altered residues in the double mutant AR significantly reduce unfavorable steric interactions of these bound ligands in the D-ring region (i.e., interactions that normally prevent or inhibit helix 12 closure and agonism in the wild-type AR) (Matias et al. 2002). Particularly with regard to agonistic activity

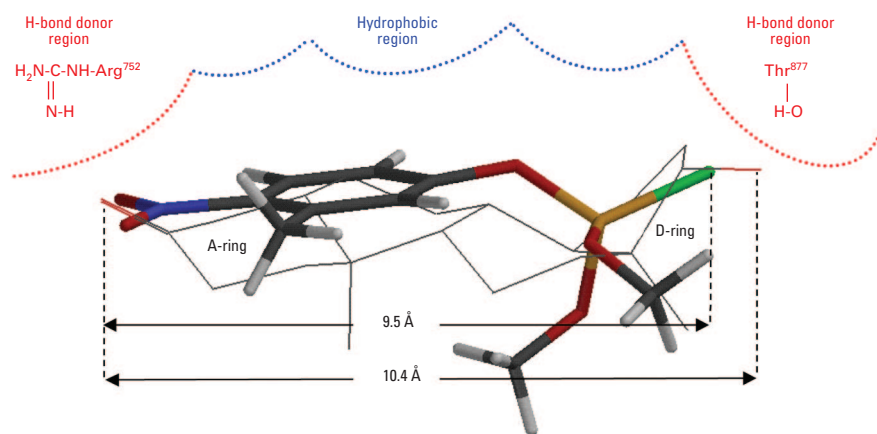


Figure 4. Schematic representation of the androgen receptor ligand binding domain comparing proposed binding configurations of DHT (thin-line structure) and fenitrothion (thick-line structure), with distances between H-bond centers indicated for each.

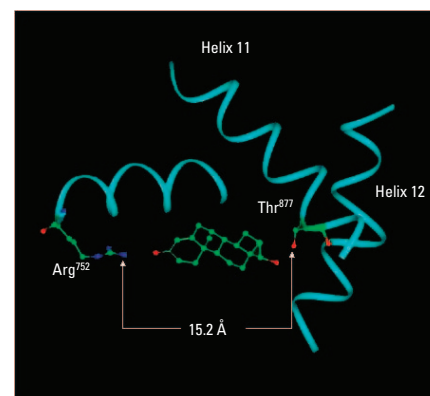


Figure 5. Representation of the DHT-bound AR-LBD derived from crystal structure coordinates (Matias et al. 2000; Sack et al. 2001), showing the distance between putative H-binding sites on the residues Arg⁷⁵² and Thr⁸⁷⁷, and in relation to the locations of helices 11 and 12. [Figure generated using Insight 2000 (Accelrys, Inc), kindly provided by S. Keenan.]

of hydroxyflutamide under different experimental conditions, these results indicate further modulation and sensitivity of agonism to subtle steric and conformational influences in the D-ring binding region.

The implication of these arguments is that fenitrothion, because it is capable of acting either as an AR antagonist or agonist, depending on the experimental conditions (competitive or noncompetitive binding), is capable of satisfying all three binding requirements for AR-LBD interaction, but only under selected conditions. With no reported evidence to suggest allosteric binding mechanisms for AR activity, and evidence pointing to the ability of nonsteroidal AR ligands to effectively bind in the AR-LBD (Marhefka et al. 2001), we posit that AR agonism is only observed at high doses of ligand due to the probabilistic nature of the AR binding interaction. The probability of initially encountering the AR has a first-order dependence on dose of ligand (P_1). Second is the probability of being drawn into the AR-LBD cavity and binding in some fashion (P_2); fenitrothion likely has a lower P_2 than the natural DHT ligand due to sub-optimal binding affinity in the A-ring and central hydrophobic regions. Finally, the conformational flexibility of fenitrothion and other nonsteroidal AR ligands introduces a third probabilistic component. In the partially AR-bound state, fenitrothion has a less than unit probability (P_3) of achieving the stretched configuration for optimal H-bonding interaction in the C17 β hydroxyl region that is presumed necessary for AR transcriptional activity; achieving this configuration will depend on the local molecular dynamics forces within the AR-LBD. Hence, with sub-optimal binding (i.e., satisfying only two of the three binding elements), antagonism is the predicted and more probable outcome (the product of the fractional probabilities $P_c \times P_1$). Because agonism requires the third binding element also be achieved (i.e., the “near-10 Å polar interactions rule”), it is a less probable event (i.e., $P_c \times P_2 \times P_3$). Because overall probabilities for observing either antagonism or agonism increase with increasing dose of fenitrothion (i.e., P_c), the overall probability of observing agonism, if achievable, will be greatest at the highest fenitrothion doses. The implications for hazard assessment are that nonsteroidal chemicals should be screened for all three of the binding elements for AR-LBD interaction, and those compounds predicted to satisfy all three elements and effect transcription (i.e., act as agonists) may be of greater concern.

A final point concerns the comparison of different quantitative and qualitative measures of androgenic activity across a variety of assays and test systems for the purposes of generating SAR hypotheses and prediction models. If

quantitative activities (e.g., relative binding affinities) are used in deriving a potency prediction model, clearly these must be for a validated and uniform measure of androgenic activity. Unfortunately, the available data are not uniform in this sense and represent a variety of measures across a variety of test systems (e.g., Singh et al. 2000). This accounts for the relatively limited data sets used in previously reported QSAR models for predicting androgenic activity of environmental chemicals. For the purposes of qualitative identification of important SAR features and structural elements relative to AR activity, however, we believe that comparisons can be made across diverse data sets provided that one uses only relative information extracted from within those data sets (e.g., in considering the structural features that distinguish agonist from antagonist activity among the HPAs) (Tucker et al. 1988). Prior SAR investigations aimed at understanding and optimizing the activity of nonsteroidal antiandrogens contain a wealth of potentially useful information (Singh et al. 2000; Teutsch et al. 1994; Tucker et al. 1988). These data can and should be considered in efforts to generate useful hypotheses and appropriate model constraints for screening of environmental compounds for potential androgenic activity.

The results of this study have important implications for future attempts to construct SAR models for predicting potential androgenic activity. First, conformational flexibility of potential ligands should be considered in light of the determining constraints for optimal AR interaction—namely, the ability to achieve a suitable distance separation of polar interaction groups (approximately 10 Å) at low energy cost. If conformational flexibility is not taken into account, AR homology models and other types of SAR screens may fail to detect the potential for AR binding interaction. Second, the demonstrated AR antagonist activities of fenitrothion, methylparathion (3), and ethylfenitrothion (5) provide compelling evidence that the thiophosphonyl group bears sufficient analogy to the hydroxylamide moiety of hydroxyflutamide as to similarly satisfy AR-LBD binding elements for both antagonism and agonism. This is despite the different H-bonding interactions predicted for the thiol H-bond acceptor in fenitrothion versus the hydroxyl H-bond donor in hydroxyflutamide or DHT within the AR-LBD. Third, the results reported in Table 1 for a series of organophosphorus-like chemicals point to structural features that either determine or enhance the ability of these compounds to act as AR antagonists. These results expand our knowledge of structural binding elements that can be accommodated by the AR-LBD and that should be considered in future efforts to develop general screens for AR activity of environmental chemicals.

REFERENCES

- Anstead GM, Carlson KE, Katzenellenbogen JA. 1997. The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site. *Steroids* 62:268–303.
- Buchanan G, Yang M, Harris HM, Nahm HS, Han G, Moore N, et al. 2001. Mutations at the boundary of the hinge and ligand binding domain of the androgen receptor confer increased transactivation function. *Mol Endocrinol* 15:46–56.
- Brzozowski AM, Pike ACW, Dauter Z, Hubbard RE, Bonn T, Engstrom O, et al. 1997. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* 389:753–758.
- Doesburg P, Kuil CW, Berrevoets CA, Steketeer K, Faber PW, Mulder E, et al. 1997. Functional *in vivo* interaction between the amino-terminal, transactivation domain and the ligand binding domain of the androgen receptor. *Biochemistry* 36:1052–1064.
- Eto M, ed. 1974. *Organophosphorus Pesticides: Organic and Biological Chemistry*. New York: CRC Press.
- Fang H, Tong W, Shi LM, Blair R, Perkins R, Branham W, et al. 2001. Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. *Chem Res Toxicol* 14:280–294.
- Goldstein RA, Katzenellenbogen JA, Luthey-Schulten ZA, Seielstad DA, Wolynes PG. 1993. Three-dimensional model for the hormone binding domains of steroid receptors. *Proc Natl Acad Sci USA* 90:9949–9953.
- Gottlieb B, Leivaslaiho H, Beitel LK, Lombroso R, Pinsky L, Trifiro M. 1998. The androgen receptor gene mutations data base. *Nucleic Acids Res* 26:234–238.
- Gray LE Jr, Ostby J, Cooper RL, Kelce WR. 1999a. The estrogenic and antiandrogenic pesticide methoxychlor alters the reproductive tract and behavior without affecting pituitary size or LH and prolactin secretion in male rats. *Toxicol Ind Health* 15:37–47.
- Gray LE Jr, Ostby J, Monosson E, Kelce WR. 1999b. Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. *Toxicol Ind Health* 15:48–64.
- Gray LE Jr, Wolf C, Lambright C, Mann P, Price M, Cooper RL, et al. 1999c. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, *p,p'*-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicol Ind Health* 15:94–118.
- Ing NH, O'Malley BW. 1995. The steroid hormone receptor superfamily-molecular mechanisms of action. In: *Molecular Endocrinology: Basic Concepts and Clinical Correlations* (Weintraub B, ed). New York: Raven Press, 195–215.
- Kelce WR, Monosson E, Gamcsik MP, Laws SC, Gray LE Jr. 1994. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. *Toxicol Appl Pharmacol* 126:276–285.
- Kelce WR, Stone CR, Laws SC, Gray LE, Kempainen JA, Wilson EM. 1995. Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. *Nature* 375:581–585.
- Kenakin T. 1993. Competitive antagonism. In: *Pharmacologic Analysis of Drug-Receptor Interaction* (Kenakin T, ed). New York: Raven Press, 279–322.
- Loughney DA, Schwender CF. 1992. A comparison of progestin and androgen receptor binding using the CoMFA technique. *J Comput Aided Mol Des* 6:569–581.
- Maness SC, McDonnell DP, Gaido KW. 1998. Inhibition of androgen receptor-dependent transcriptional activity by DDT isomers and methoxychlor in HepG2 human hepatoma cells. *Toxicol Appl Pharmacol* 151:135–142.
- Marhefka CA, Moore BM, Bishop TC, Kirkovsky L, Mukherjee A, Dalton J, et al. 2001. Homology modeling using multiple molecular dynamics simulations and docking studies of the human androgen receptor ligand binding domain bound to testosterone and nonsteroidal ligands. *J Med Chem* 44:1729–1740.
- Matias PM, Carrondo MA, Coelho R, Thomaz M, Zhao XY, Wegg A, et al. 2002. Structural basis for the glucocorticoid response in a mutant human androgen receptor (AR^{R272}) derived from an androgen-independent prostate cancer. *J Med Chem* 45:1439–1446.
- Matias PM, Donner P, Coelho R, Thomaz M, Peixoto C, Macedo S, et al. 2000. Structural evidence for ligand specificity in the binding domain of the human androgen receptor.

- Implications for pathogenic gene mutations. *J Biol Chem* 275:26164–26171.
- McIntyre BS, Barlow NJ, Wallace DG, Maness SC, Gaido KW, Foster PMD. 2000. Effects of *in utero* exposure to linuron on androgen-dependent reproductive development in the male Crl:CD(SD)BR rat. *Toxicol Appl Pharmacol* 167:87–99.
- McKinney JD, Waller CL. 1994. Polychlorinated biphenyls as hormonally active structural analogues. *Environ Health Perspect* 102:290–297.
- Mekenyan OG, Ivanov JM, Karabunarliev SH, Bradbury SP, Anklew GT, Karcher W. 1997. COREPA: A new approach for the elucidation of Common Reactivity Patterns of chemicals. I. Stereoelectronic requirements for androgen receptor binding. *Environ Sci Technol* 31:3702–3711.
- Monosson E, Kelce WR, Lambright C, Ostby J, Gray LE Jr. 1999. Peripubertal exposure to the antiandrogenic fungicide, vinclozolin, delays puberty, inhibits the development of androgen-dependent tissues, and alters androgen receptor function in the male rat. *Toxicol Ind Health* 15:65–79.
- Nishizawa Y, Nakagawa M, Suzuki H, Sakamoto H, Mizutani T. 1961. Studies on the organophosphorus insecticides. VI. Development of new low toxic phosphorus insecticides. *Agricul Biol Chem* 25:597–610.
- Ostby J, Kelce WR, Lambright C, Wolf CJ, Mann P, Gray LE Jr. 1999. The fungicide procymidone alters sexual differentiation in the male rat by acting as an androgen-receptor antagonist *in vivo* and *in vitro*. *Toxicol Ind Health* 15:80–93.
- Pike ACW, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, et al. 1999. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *EMBO J* 18:4608–4618.
- Poujol N, Wurtz JM, Tahiri B, Lumbroso S, Nicolas JC, Moras D, et al. 2000. Specific recognition of androgens by their nuclear receptor. A structure-function study. *J Biol Chem* 275:24022–24031.
- Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, et al. 1995. Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. *Nature* 378:681–689.
- Sack JS, Kish KF, Wang C, Attar RM, Kiefer SE, An Y, et al. 2001. Crystallographic structures of the ligand-binding domains of the androgen receptor and its T877A mutant complexed with the natural agonist dihydrotestosterone. *Proc Natl Acad Sci USA* 98:4904–4909.
- Safe SH, Zacharewski T. 1997. Organochlorine exposure and risk for breast cancer. *Prog Clin Biol Res* 396:133–145.
- Singh MS, Gauthier S, Labrie F. 2000. Androgen receptor antagonists (antiandrogens): structure activity relationships. *Curr Med Chem* 7:211–247.
- Stewart JJP. 1998. Optimization of parameters for semiempirical methods. I. Method. *J Comp Chem* 10:209–220.
- Sultan C, Lumbroso S, Poujol N, Belon C, Boudon C, Lobaccaro JM. 1993. Mutations of androgen receptor gene in androgen insensitivity syndromes. *J Steroid Biochem Mol Biol* 46:519–530.
- Tamura H, Maness SC, Reischmann KP, Dorman D, Gray LE Jr, Gaido KW. 2001. Fenitrothion: androgen receptor antagonism by the organophosphate insecticide fenitrothion. *Toxicol Sci* 60:56–62.
- Tanenbaum DM, Wang Y, Williams SP, Sigler PB. 1998. Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. *Proc Natl Acad Sci USA* 95:5998–6003.
- Taplin ME, Bublely GJ, Ko YJ, Small EJ, Upton M, Rajeshkumar B, et al. 1999. Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. *Cancer Res* 59:2511–2515.
- Teutsch G, Goubet F, Battmann T, Bonfils A, Bouchoux F, Cerede E. 1994. Non-steroidal antiandrogens: synthesis and biological profile of high-affinity ligands for the androgen receptor. *J Steroid Biochem Mol Biol* 48:111–119.
- Tong W, Perkins R, Strelitz R, Collantes ER, Keenan S, Welsh WJ, et al. 1997a. Quantitative structure-activity relationships (QSARs) for estrogen binding to the estrogen receptor: predictions across species. *Environ Health Perspect* 105:1116–1124.
- Tong W, Perkins R, Xing L, Welsh WJ, Sheehan DM. 1997b. QSAR models for binding of estrogenic compounds to estrogen receptor alpha and beta subtypes. *Endocrinology* 138:4022–4025.
- Tong W, Lewis DR, Perkins R, Chen Y, Welsh WJ, Goddette DW, et al. 1998. Evaluation of quantitative structure-activity relationship methods for large-scale prediction of chemicals binding to the estrogen receptor. *J Chem Inf Comput Sci* 38:669–677.
- Tucker H, Crook JW, Chesterson GJ. 1988. Nonsteroidal antiandrogens. Synthesis and structure-activity relationships of 3-substituted derivatives of 2-hydroxypropionanilides. *J Med Chem* 31:954–959.
- Waller CL, Juma BW, Gray LE Jr, Kelce WR. 1996a. Three-dimensional quantitative structure-activity relationships for androgen receptor ligands. *Toxicol Appl Pharmacol* 137:219–227.
- Waller CL, Oprea TI, Chae K, Park HK, Korach KS, Laws SC, et al. 1996b. Ligand-based identification of environmental estrogens. *Chem Res Toxicol* 9:1240–1248.
- Wiese TE, Brooks SC. 1994. Molecular modeling of steroidal estrogens: novel conformations and their role in biological activity. *J Steroid Biochem Mol Biol* 50:61–73.
- Wolff MS, Toniolo PG. 1995. Environmental organochlorine exposure as a potential etiologic factor in breast cancer. *Environ Health Perspect* 103(suppl 7):141–145.
- Wong C, Kelce WR, Sar M, Wilson EM. 1995. Androgen receptor antagonist versus agonist activities of the fungicide vinclozolin relative to hydroxyflutamide. *J Biol Chem* 270:19998–20003.
- Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D, et al. 1996. A canonical structure for the ligand-binding domain of nuclear receptors. *Nat Struct Biol* 3:206.
- Veldscholte J, Ris-Stalpers C, Kuiper GG, Jenster G, Berrevoets C, Claassen E, et al. 1990. A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. *Biochem Biophys Res Commun* 173:534–540.