to the metabolites, luteolin or quercetin, while tomatine acts via a non-EcR pathway. Neither coumestrol nor genistein inhibited EcR-dependent gene transcription. In plants, flavones are signaling molecules and cause nitrogen-fixing bacteria to form root nodules. The bacterial NodD protein which regulates this symbiosis has two ligand-binding domains which are similar to that of human estrogen receptor (hERα). NodD, EcR and ER all interact with flavones, which may have evolutionary significance.

1786 APPLICATION OF THE CALUX BIOASSAY FOR DETECTION OF PHARMACOLOGICAL AGENTS WHICH ACTIVATE THE AH RECEPTOR SIGNALING SYSTEM.

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The Ah receptor (AhR) is a ligand dependent transcription factor that mediates the majority of the biochemical and toxicological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related chemicals. Utilizing two recently developed AhR-based screening systems, the CALUX (Chemically Activated LUciferase eXpression) cell bioassay (employing cell lines stably transfected with a TCDD/AhR-responsive firefly luciferase reporter gene) and an inducible AhR-dependent DNA binding (gel shift) assay we have begun large scale screening of chemicals and chemical mixtures for their ability to activate the AhR signaling pathway. We have identified several pharmacologically-relevant chemicals including imidazoline receptor ligands (idazoxan, guanabenz and epinephrine) and benzimidazole drugs (omeprazole, albendazole and thiabendazole) which induced luciferase activity in several different CALUX cell lines. Although some of the imidazoline receptor ligands were also potent activators of AhR transformation and DNA binding in vitro, others were essentially inactive; all of the benzimidazoles were significantly less potent in the in vitro bioassay. These results, combined with those obtained using other chemicals reveal significant discrepancies between the ability of a chemical to activate the AhR in vitro and in intact cells. This clearly demonstrates the serious limitation of only using in vitro AhR-based bioassays in chemical screening approaches. (Supported by NIEHS (ES08372, ES07685, ES04699).)

1787 EFFECTS OF PHYTOCHEMICALS ON MCF-7 CELL GROWTH AND ESTROGEN RECEPTOR BINDING.

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Recent attention has focused on the isoflavanoids in soya and the flavanoids in tea as possible mediators of carcinogenesis. In this study, we measured the binding properties of three isoflavanoid compounds found in soya- genistein, daidzein and coumestrol-, and two flavanoid compounds found in tea- catechin and epicatechin. The effects of these phytochemicals on the cell proliferation of estrogen-responsive MCF-7 breast cancer cells was also studied. Receptor binding to human estrogen receptor (ER) was measured with a Beacon 2000 fluorescence polarization system (PanVera, Inc, Madison, WI). Expressed, purified ER (PanVera, Inc, Madison, WI) was preincubated for 30 min with an optimal concentration of a fluorescent estrogen analog (EF, PanVera, Inc, Madison, WI) and then concentrations of the test compound (10^{-9} to 10^{-4} M) were added to the ER-EF complex. competitive binding of the phytochemicals was measured as a decrease in polarized fluorescence after 2 hrs. MCF-7 cell proliferation was determined by seeding 10^4 cells into 24 well plates and exposing the cells to varying concentrations of the test compounds in the presence and absence of estradiol for 5 days. On the sixth day, the cells were fixed and the effects on cell growth determined by measuring total protein in the cells as compared to the protein in cells incubated with an optimum concentration of estradiol or with vehicle alone. Genistein and coumestrol displaced EF from ER in the range of 10 μM while catechin and epicatechin displaced EF from ER in the range of 100 μM. Coumestrol stimulated MCF-7 cell growth at concentration ≥10 nM and genistein stimulated MCF-7 cell growth at concentration ≥100 nM, but both catechin and epicatechin strongly inhibited the growth of MCF-7 cells at all concentrations tested (10^{-10} M to 10^{-5} M). The effects of these phytochemicals on the growth of estrogen-responsive MCF-7 breast cancer cell, therefore, do not correlate with their competitive binding to the human ER.

1788 OPTIMAL pH AND SOLVENT CONCENTRATION FOR AN *IN VITRO*, HUMAN ESTROGEN RECEPTOR GENE TRANSACTIVATION ASSAY.

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The estrogen receptor (ER) gene transactivation assay is currently being considered as a tool for large scale screening of materials from a wide range chemical classes (e.g., strong acids/bases). Therefore, the goal of this study was to determine optimal ranges for pH and solvent (dimethylsulfoxide; DMSO) concentration in this assay. MCF-7 cells were transfected with a chimeric Gal4-ER construct and a Gal4-regulated lucerifase reporter gene, then were exposed overnight to a range of 17β-estradiol (E2) concentrations (10^{-12} to 10^{-7} M), each tested at various pH levels (pH 6.8, 7.3, 7.5, 7.8, 8.0, 8.2, 8.8) or DMSO concentrations (0.1, 0.5, 1.0% v/v). At the end of incubation, the cells were lysed and the lysate assayed for luciferase activity. Maximal E2-induced reporter activity (25-40 fold relative to vehicle controls) occurred at pH 7.8, with a marked decrease in E2-induced activity at pH ≤ 7.5 or ≥ 8.0 (pH effect significant at $p<0.001$). E2-induced reporter activity tended be highest at a DMSO concentration of 0.1%, and lowest at 1.0% DMSO, while 0.5% DMSO produced somewhat variable responses across studies. These results show that pH is a critical variable in the conduct and interpretation of this ER reporter gene assay, and also suggest that DMSO concentration should not exceed 0.1% for optimal assay performance. (*Funded by The Chemical Manufacturers Association (Arlington, VA).*)

1789 USE OF A RECOMBINANT HUMAN BREAST CANCER CELL LINE, MVLN, TO SCREEN FOR ESTROGENIC ACTIVITY AMONG STRUCTURALLY-RELATED ALKYLPHENOLIC COMPOUNDS.

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Alkylphenolic compounds are used in great volumes, primarily in the manufacture of surfactants. Several of these compounds have been observed to possess weakly estrogenic activity. The purpose of the current study was to utilize the cell line, MVLN, which is a MCF-7 human breast cancer cell line that is stably transfected with a luciferase reporter under the transcriptional control of the estrogen receptor (ER) to determine relative estrogenic potencies for several alkylphenolic compounds compared to 17-β-estradiol (E_2) as a standard reference compound. Individual test compounds were tested for both their ability to produce a statistically significant response relative to the solvent control and their ability to elicit a full dose-response curve. Each test agent was tested in the absence of E_2 and in the presence of an EC_{50} concentration of E_2 (10 pM). The most potent test agent was tecnical nonylphenol (a mixture of ring and chain isomers) with a relative potency of 0.0001 compared to E_2. As expected, significant structure-activity relationships were observed. The number and position of substituents on the phenolic ring, length of carbon chain, and branched versus straight carbon chains were found to be important determinants of activity. A test agent with a straight carbon chain, 4-*n*-nonylphenol, was found to be inactive as an estrogen agonist up to 5 μM and was cytotoxic at greater concentrations. All of the alkylphenolic compounds that were tested, except technical nonylphenol, were either inactive as estrogen agonists or were weakly estrogenic. The MVLN cell line was found to be a useful screening assay that is sensitive and specific for estrogenic chemicals.

1790 SIMILARITIES BETWEEN ACETAMINOPHEN- AND ESTRADIOL-INDUCED PROLIFERATION OF CULTURED, ESTROGEN-RESPONSIVE BREAST CANCER CELLS.

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Studies in this laboratory have shown that acetaminophen, a widely used analgesic/antipyretic, induces DNA synthesis and proliferation in estrogen-responsive (ER+) but not estrogen-nonresponsive (ER-) cultured, human breast cancer cells. Like estradiol, acetaminophen contains a *p*-phenol moiety; this moiety appears to influence estradiol- and xenoestrogen-induced ER+ cell proliferation. Thus, the first goal of this research was to determine

the importance of the *p*-phenol moiety in acetaminophen-induced proliferation. To this end, effects of *p*-, *m*-, and *o*-acetamidophenol on ER+ (MCF7 and T47D) cell proliferation and on % cells in S (DNA synthesis) phase of the cell cycle were determined. Therapeutic concentrations of *p*-acetamidophenol (~ 0.1 mM) significantly increased proliferation, and the relative order of potency was *p*- > *m*- > *o*-acetamidophenol. These data suggest the *p*-phenol moiety is important in both estradiol- and acetaminophen-induced ER+ cell proliferation. The second goal of this research was to establish if estrogen receptor pathways are involved in mediating the acetaminophen-induced proliferation of ER+ cells. Two antiestrogens (ICI 182,780 and 4-hydroxytamoxifen) were tested in 2 ER+ (MCF7, T47D) and 1 estrogen-nonresponsive, ER- (MDA-MB-231) cell lines. Effects of antiestrogens on acetaminophen-induced proliferation were assessed by flow cytometry (% S phase cells) and by cell counting. In MCF7 and T47D cells (but not in MDA-MB-231 cells), both antiestrogens inhibited proliferation induced by acetaminophen and by estradiol. These data also suggest that there are similarities between estradiol- and acetaminophen-induced proliferation. Furthermore, estrogen receptors may directly or indirectly mediate the acetaminophen-induced proliferation of ER+ cells.

1791 ROLE OF THE RAT SPLICE VARIANT OF ESTROGEN RECEPTOR BETA IN ESTROGEN SIGNALLING; IMPLICATIONS FOR ENDOCRINE DISRUPTION TESTING.

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We have analysed the functional activity of an isoform of the estrogen receptor beta (ERβ) (found in rat and mouse) which has an 18 amino-acid insert in the ligand binding domain (ERβ2; ^{320}SSEDPHWHVAQMKSAAPR^{338}). The ERβ2 is capable of binding to the vitellogenin estrogen response element as demonstrated by electrophoretic mobility shift assays. The ability of ERβ2 to activate transcriptionally a reporter plasmid (consisting of a consensus vitellogenin ERE cloned upstream of a minimal SV40 promoter driving luciferase) when transiently transfected into Cos-1 cells was examined. The reporter in cells transfected with ERβ alone was activated in a dose-dependent manner by stimulation with estradiol-17β (10^{-12} - 10^{-6} M). In contrast, estradiol failed to activate the reporter in cells transfected with ERβ2 alone. Of all ligands tested to date, only the synthetic estrogen diethyl-stilbestrol (DES) was able consistently to activate ERβ2 in this assay. In addition, when cells were co-transfected with a constant amount of ERβ and increasing amounts of ERβ2 there was a significant decline in reporter activation by estradiol. Using *in situ* hybridization and RTPCR analyses, we have demonstrated expression of ERβ and ERβ2 in the rat prostate, ovary and pituitary while ERβ2 mRNA is not detectable in the αT3-1 rat pituitary gonadotrope specific cell line. These data provide evidence for an isoform of ERβ which may act as a negative regulator of ERβ function. The differences in relative abundance of these two receptors in different tissues, and the species specific nature of ERβ2 may play a role in determining species and tissue-specific responses to estrogenic substances.

1792 NEW DEVELOPMENTS IN A HAZARD IDENTIFICATION ALGORITHM FOR HORMONE RECEPTOR LIGANDS.

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Recently we described the Common REactivity PAttern (COREPA) technique to screen data sets of diverse structures for their ability to serve as ligands for steroid hormone receptors (Environ. Sci. Technol. 31:3702-3711). The approach identifies and quantifies similar global and local stereoelectronic characteristics associated with active ligands through a comparison of energetically-reasonable conformer distributions for selected descriptors. For each stereoelectronic descriptor selected, discrete conformer distributions from a training set of ligands are evaluated and parameter ranges common for conformers from all the chemicals in the training set identified. The use of discrete partitions of parameter ranges to define common reactivity patterns can, however, influence the outcome of the algorithm. To address this limitation, the method has been extended by approximating continuous conformer distributions as probability distributions. The COREPA-Continuous (COREPA-C) algorithm assesses the common reactivity pattern of biologically similar molecules in terms of a product of probability distributions, rather than a collection of common population ranges determined by examination of discrete partitions of a distribution. To illustrate the algorithm, common reactivity patterns based on interatomic distance and charge on heteroatoms were developed and evaluated using a set of 28 androgen receptor ligands. Using receptor binding affinity as the measure of biological activity, the algorithm successfully discriminated 'active' from 'non-active' ligands. Notable attributes of the COREPA-C algorithm include flexibility in establishing stereoelectronic descriptor criteria for identifying active and non-active compounds and the ability to quantify three-dimensional chemical similarity without the need to predetermine a toxicophore or align compounds(s) to a lead ligand.

1793 MOLECULAR MODELING SIMULATIONS OF RECEPTOR-LIGAND INTERACTIONS FOR ENVIRONMENTAL ESTROGENS.

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Molecular modeling and molecular dynamics have been used to study the interaction of various environmental estrogens (EEs) with the human estrogen receptor α (hERα). EEs are endocrine disrupting chemicals (EDCs) that interfere with the normal function of ER in the effected tissues of exposed species. The modeling was based on the 3-D structure of the ligand binding domain of the hERα as recently determined by X-ray crystallographic studies from two different laboratories. The available structures include two of hERα with estradiol-17β and one of hERα with the antiestrogen raloxifene. The purpose of this study was to develop an understanding of the possible ER binding modes of nonsteroidal environmental chemicals that are known to have the capacity to displace estradiol-17β binding *in vitro*. Ligand-receptor docking studies were performed with the classic xenoestrogens DES, DDT isomers, methoxychlor, HPTE, chlordecone (kepone), t-octylphenol and bisphenol A. Each ligand-receptor model was subjected to a molecular dynamics annealing protocol and energy minimization to achieve a final binding conformation and orientation. The relative contribution of the van der Waals, electrostatic and hydrogen bonding energies to the total potential energy of each complex was determined and compared to reported *in vitro* binding affinities. An effort was also made to characterize ligand-receptor interactions that may be responsible for the relative differences in ER affinity among these xenoestrogens. These studies have resulted in the formulation of a ligand-receptor model which addresses the disparate binding capacities of various EDC's. Furthermore, these "virtual" receptor binding studies may also be of general utility as a component of endocrine disruptor screening and testing protocols.

1794 ENANTIOMER SELECTIVE ESTROGEN ACTIVITY OF o,p'-DDT AND o,p-DDD OPTICAL ISOMERS.

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Racemic mixtures of the chiral pesticides o,p'-DDT and o,p'-DDD have previously been described as endocrine disrtuping chemicals with estrogen activity. In this report, we account the estrogen activity of the individual enantiomers of o,p'-DDT and o,p'-DDD. Optically pure samples as well as racemic mixtures were evaluated for estrogen receptor (ER) binding and reporter gene activity in mammalian cells. Binding assays utilized recombinant ER and were performed at 20ûC using the PanVera fluorescence polarization method. Reporter gene assays employed MVLN cells which are MCF-7 human breast cancer cells containing ER that have been stably transfected with an estrogen responsive vit-Luc construct. All racemic mixtures and optically pure samples tested were found to have some capacity to bind the ER as well as induce MVLN reporter gene activity at concentrations up to 10 μM. It was also shown that the MVLN activity of each test compound was inhibited by the antiestrogen ICI-182,780. However, while both enantiomers and the racemic mixture of o,p'-DDD maintained minimal estrogen activity (EC_{50} from 3000nM to 10000 nM), the R or (-) enantiomer of o,p'-DDT displayed enhanced ER binding activity and was found be 25 times more potent in the MVLN assay than the S or (+) enantiomer (EC_{50} of 250 nM vs. 6500 nM respectively). This study highlights the importance of resolving the optical isomers of chiral compounds for both endocrine disruptor characterization studies as well as environmental monitoring. Furthermore, this study suggests that the enantiomer specificity of certain chiral compounds my be utilized to develop optically pure pesticides and other industrial chemicals with decreased endocrine activity.

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshop, roundtable, and poster sessions of the 38th Annual Meeting of the Society of Toxicology, held at the Ernest N. Morial Convention Center, New Orleans, Louisiana, March 14-18, 1999.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 419.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 444.

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