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Estrogenic Activity of Chemical Mixtures: Is There Synergism?

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Public and legislative concern that chemicals in our environment may be affecting human health by mimicking estrogen and disrupting normal endocrine function has fueled the development of a number of in vitro assays to screen chemicals for estrogenic activity. These assays have resulted in identification of a rapidly expanding list of chemicals with potential estrogenic activity. The low potency of most of these chemicals relative to estradiol suggests that they may have little effect on endocrine function at environmentally relevant doses. However, binary combinations of weakly estrogenic organochlorine pesticides such as endosulfan, dieldrin, toxaphene, and chlordane were recently reported to result in synergistic responses. In a yeast-based estrogen receptor assay, for example, combinations of these chemicals were 100- to 1600-fold greater than any chemical alone. Synergistic interactions between two weakly estrogenic, hydroxylated polychlorinated biphenyls were also observed. Such synergistic interaction of weakly estrogenic chemicals obviously has profound environmental and regulatory impact. In collaboration with three other laboratories, the Chemical Industry Institute of Toxicology (CIIT) reassessed the potential synergistic interactions of these chemicals using the following in vivo and in vitro estrogen-responsive assays: induction of uterine wet weight, progesterone receptor levels and uterine peroxidase activity in the immature female mouse; induction of cell growth in MCF-7 human breast cancer cells; induction of reporter gene activities in two yeast-based assays that expressed either the human or mouse estrogen receptor; induction of reporter gene activity in HepG2 human hepatoma cells; and competitive estrogen receptor binding in MCF-7 cells and to the mouse uterine estrogen receptor. In contrast to previously reported findings, synergistic interactions were not observed for these nine different estrogen-responsive assays. Thus synergism of weakly estrogenic chemicals is not universally observed, and the potential human health risk associated with exposure to organochlorine mixtures needs to be further evaluated to understand any possible impact.

The steroid hormone 17β-estradiol and other estrogens play an important role in regulating the growth, differentiation, and function of reproductive tissues, including the uterus, vagina, ovary, oviduct, and mammary gland. Estrogen also has important sites of action in the pituitary, hypothalamus, and specific brain regions and exerts crucial actions on other tissues such as bone, liver, and the cardiovascular system. Estrogen is involved in sexual development, and exposure to high estrogen concentrations during critical periods of development can lead to teratogenic and carcinogenic lesions in the reproductive tracts of humans. Furthermore, estrogen is implicated in the initiation and progression of breast, ovarian, endometrial, and prostate cancers.

The biological actions of estrogen are mediated by high-affinity receptor proteins located within target cells (Ing and O'Malley, 1995). In the absence of estrogen, these estrogen receptors reside in an inactive conformation. The interaction of estrogen with its receptor induces a conformational change in the receptor, thereby initiating a cascade of events that ultimately leads to the association of an estrogen receptor dimer with specific DNA sequences within the regulatory regions of estrogen-responsive genes (Figure 1). The altered expression of these estrogen-responsive genes produces the myriad effects associated with this steroid hormone.

Other chemicals that can bind to the estrogen receptor and mimic the actions of estrogen have also been identified. These chemicals include natural products such as coumestrol and genistein; pharmaceuticals such as diethylstilbestrol, ethynylestradiol, and tamoxifen; and industrial chemicals such as bisphenol A, *p*-nonylphenol, and *o,p'*-DDT. The observation that industrial chemicals can mimic the actions of estrogen has raised public concern that chemicals in our environment are affecting human

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Estrogenic Activity (from page 1)

health by disrupting normal endocrine function through interaction with the estrogen receptor; this disruption leads to altered hormonal balance, which affects reproductive capacity, infertility, endometriosis, and cancers of the breast, uterus, and prostate (Sharpe and Skakkebaek, 1993; Colborn, 1995; vom Saal, 1995).

To date, the industrial chemicals labeled as estrogenic are much less potent than estradiol. Whether exposure to these weakly active chemicals at environmentally relevant concentrations poses a threat to human health remains to be determined. However, a recent study by Arnold and coworkers (1996) has heightened concern regarding the potential adverse effects of exposure to mixtures of weakly estrogenic chemicals. The authors reported synergistic interactions between binary mixtures of the pesticides endosulfan, dieldrin, toxaphene, and chlordane in competitive estrogen receptor binding assays and in an estrogen-responsive assay in yeast. In the yeast-based assay, for example, dieldrin and toxaphene alone induced less than 50% of maximal reporter activity at concentrations of 3.3×10^{-5} M, whereas the EC_{50} value for 17β-estradiol (E2) was 10^{-10} M. In yeast cells treated with an equimolar mixture of dieldrin plus toxaphene, however, the EC_{50} value was 2.1×10^{-7} M, a greater than 150-fold synergistic response. Moreover, some of these interactions involved a synergistic response of up to 1600-fold. Less dramatic synergistic interactions (5- to 10-fold) between two weakly estrogenic, hydroxylated polychlorinated biphenyl congeners were also observed in the yeast assay and in human endometrial cancer cells. Based on these data, Arnold et al. (1996) suggested "that the estrogenic potency of some environmental chemicals, when tested singly, may be underestimated."

The report by Arnold et al. (1996) has obvious scientific, regulatory, and public health implications (Kaiser, 1996; Simons, 1996). Together with the recent passage of amendments to the Food Quality Protection Act and the Water Safety Act, which will require screening of chemicals for estrogenic activity, the report has raised the daunting prospect that chemicals may now need to be tested in combinations. In addition, chemicals that were once thought to be of little concern because of their relatively weak estrogenic potency may now need to be reconsidered in terms of their synergistic potential.

The results by Arnold et al. have subsequently been challenged by our laboratories and others (Ashby et al., 1997; Ramamoorthy et al., 1997a; Ramamoorthy et al., 1997b; Arcari and Gierthy, 1997; Weise et al., 1997). In this article, we describe the collaborative effort between the Chemical Industry Institute of Toxicology (CIIT) and investigators from Duke University, the National Institute of Environmental Health Sciences (NIEHS), and Texas A&M University to investigate the interactions of the organochlorine pesticides endosulfan, dieldrin, toxaphene, and chlordane and the hydroxylated polychlorinated biphenyls 2′,4′,6′,-trichloro-4-biphenylol and 2′,3′,4′,5′-tetrachloro-4-biphenylol. Results from nine different assays for estrogenicity demonstrate that the estrogenic activity with binary mixtures of these chemicals is additive. These results indicate that synergism is not a universally observed finding. While understanding the reported synergistic response remains of scientific interest, evidence is currently insufficient, based on the results from our laboratories and others (Ashby et al., 1997; Ramamoorthy et al., 1997a; Ramamoorthy et al., 1997b; Arcari and Gierthy, 1997; Weise et al., 1997), to incorporate synergism in the risk assessment of weakly estrogenic chemicals. Further evaluation and testing will be required before a definite role for synergism in mechanism-based toxicity studies can be made.

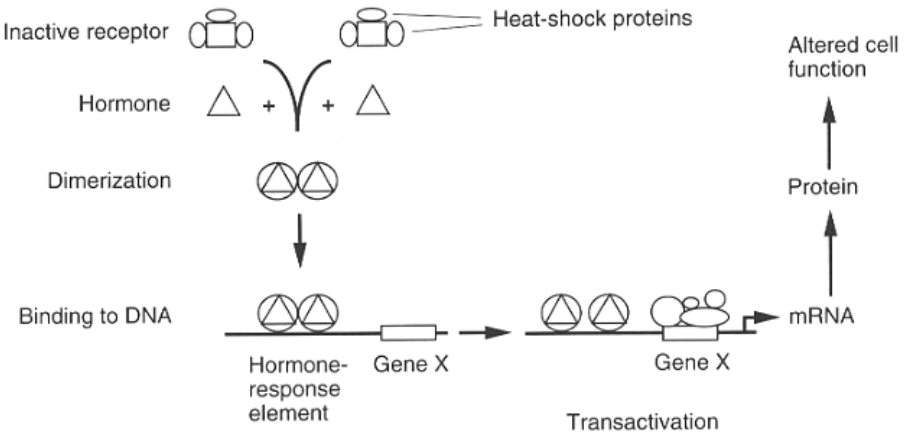

Figure 1
Molecular pathway of steroid hormone action. The biological actions of steroid hormones are mediated by high-affinity receptor proteins located within target cells. In the absence of hormone, these receptors reside in an inactive conformation in complex with heat-shock proteins. The interaction of hormone with its receptor induces a conformational change in the receptor. A cascade of events is thereby initiated that includes dissociation of heat-shock proteins and dimerization of the hormone-hormone receptor complex with another hormone-hormone receptor complex. The dimerized receptor complex binds to specific DNA sequences (hormone-response elements) within regulatory regions of hormone-responsive genes and interacts with other regulatory proteins to alter gene transcription. Changes in gene transcription lead to altered mRNA and protein concentrations, which ultimately result in a change in cellular function.

Yeast-Based Estrogen Receptor Assay

Because of their ease of growth and manipulation, the yeast strain *Saccharomyces cerevisiae* (bakers' yeast) is frequently used to express mammalian proteins. While yeasts do not have steroid receptors of their own, mammalian steroid receptors function normally when expressed in yeast (Metzger et al., 1988; McDonnell et al., 1991). As a result, yeast serves as a useful model system for studying mammalian steroid receptor function in isolation from confounding factors found in mammalian cells.

We have previously demonstrated the utility of the yeast-based estrogen receptor assay for characterizing chemical interaction with the estrogen, androgen, and progesterone receptors and have established the relative potency for a number of natural and synthetic estrogens and several industrial chemicals (Table 1) (Gaido et al., 1997). To perform a yeast-based estrogen receptor assay, yeast cells are transformed with DNA that encodes for the estrogen receptor (expression plasmid) and a reporter plasmid that contains one or more tandemly linked, estrogen-responsive elements upstream of an easily measurable reporter gene such as β-galactosidase (Gaido et al., 1997). Estrogen or other chemicals that bind to the estrogen receptor activate transcription of the β-galactosidase gene. β-Galactosidase activity is readily measured in a colorimetric assay (Figure 2). Only those chemicals that bind to the estrogen receptor and activate transcription induce β-galactosidase production. Thus the yeast-based estrogen receptor assay is a highly specific and sensitive method for assessing chemical interaction with the estrogen receptor.

We examined the estrogenic activity of the organochlorine pesticides endosulfan, toxaphene, dieldrin, and chlordane and binary mixtures of these chemicals in yeast that had been transformed with either the mouse or the human estrogen receptor. Estrogenic activities of these chemicals

were minimal in yeast transformed with the mouse estrogen receptor, and no synergistic effects were observed for their equimolar, binary mixtures (data not shown). (An equimolar mixture is an equivalent molar concentration of each chemical such that the total of the two equals the final concentration. For example, a binary mixture of dieldrin and toxaphrene with an equimolar concentration of 10 μmol will contain 5 μmol of dieldrin plus 5 μmol of toxaphene.) The potential interactions of these pesticides were also investigated in a yeast-based human estrogen receptor assay that used the same yeast strain and reporter gene construct employed by Arnold and coworkers (1996). In contrast to their study, we did not observe synergism for binary mixtures of any pesticide combination (Figure 3, left). Thus our results utilizing both the mouse and human estrogen receptors in two different yeast strains do not support the 100- to 1000-fold synergistic interaction of these chemical mixtures previously reported (Arnold et al., 1996).

McLachlan et al. (1997) suggested that the differences between our results and those reported by Arnold et al. (1996) were due to differences in estrogen receptor concentration. Reducing the level of estrogen receptor expression in the yeast assay did not have any effect on synergy, however (Figure 3, right). This lack of effect apparently negates differences in estrogen receptor concentration as a viable explanation.

Table 1
Relative Potency Ratios for Yeast-Based Estrogen Receptor Assay

Chemical	No. of experiments[a]	EC_{50}	Lower confidence limit	Upper confidence limit	Potency ratio[b]
Estradiol	32	2.25E-10	1.78E-10	2.82E-10	1.00
DES	4	3.53E-10	2.74E-10	4.54E-10	1.57
Coumestrol	5	1.74E-08	1.05E-08	2.85E-08	77.00
Estriol	3	6.16E-08	4.17E-08	8.87E-08	273.00
Dihydrotestosterone	8	4.31E-07	2.72E-07	6.69E-07	2,000.00
p-Nonylphenol	3	1.10E-06	8.24E-07	1.47E-06	5,000.00
Bisphenol A	3	3.40E-06	2.85E-06	4.08E-06	15,000.00
Nafoxidine	5	7.72E-06	4.37E-06	1.21E-05	34,000.00
Clomiphene	3	9.97E-06	7.31E-06	1.36E-05	44,000.00
ICI 164,384	3	1.45E-05	1.33E-05	1.60E-05	64,000.00
β-Sitosterol	7	4.92E-05	4.28E-05	6.06E-05	220,000.00
Testosterone	6	5.09E-05	4.40E-05	5.56E-05	226,000.00
Methoxychlor	2	1.20E-03	8.80E-04	1.68E-03	5,000,000.00
o,p'-DDT	4	1.81E-03	1.02E-03	6.57E-03	8,000,000.00
o,p'-DDD	6	3.32E-03	2.73E-03	4.07E-03	15,000,000.00
o,p'-DDE	3	5.34E-03	4.30E-03	7.51E-03	24,000,000.00

[a]Number of experiments performed for each chemical.
[b]The potency ratio is defined as the ratio of EC_{50} (chemical) over EC_{50} (estradiol).
(From Gaido et al., 1997.)

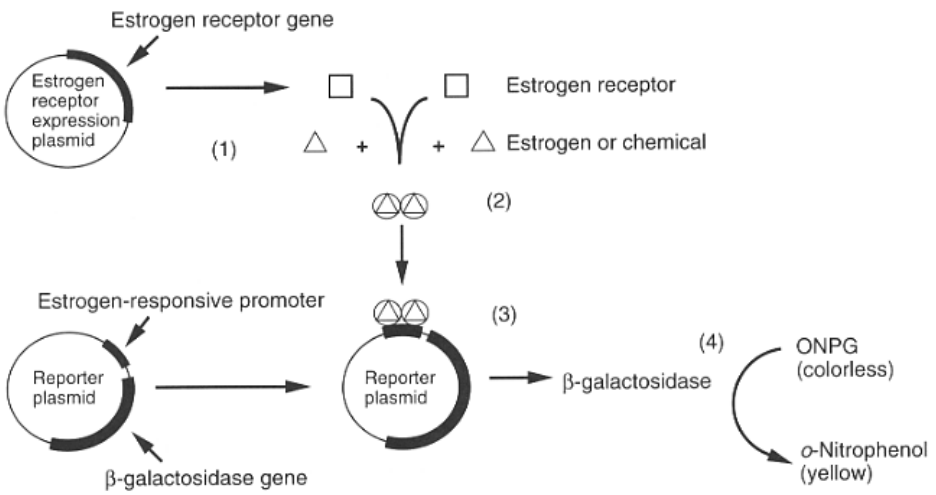

Figure 2
Yeast-based estrogen receptor assay. The human estrogen receptor is expressed from its expression plasmid in an inactive form (1). Binding of estrogen or chemical ligand to the estrogen receptor results in dimerization and activation of the receptor to a form capable of binding to estrogen-responsive elements on the promoter of the reporter plasmid (2), resulting in production of the enzyme β-galactosidase (3). β-galactosidase converts the chromogenic substrate *o*-nitrophenyl β-*o*-galactopyranoside (ONPG) into a yellow product that can be measured by absorbance (4).

Mammalian-Based Steroid Receptor Assay

To further investigate the role (if any) of receptor concentration in the estrogenic activity of chemical mixtures, we transfected various concentrations of estrogen receptor DNA into a human hepatoma cell line (HepG2). The HepG2 cell line is a widely used line derived from a liver tumor biopsy. These cells do not express steroid receptors of their own and thus serve as a useful model for expressing steroid receptors of interest for further study. Numerous studies have been published demonstrating the utility of HepG2 cells for studying receptor function (Spink et al., 1994; Tzukerman et al., 1994; Boucher and Hines, 1995; Wiebel et al., 1996; Yang et al., 1996).

The HepG2 estrogen receptor assay (Tzukerman et al., 1994) is similar to the yeast-based assay. Like the yeast, HepG2 cells are transfected with an expression plasmid that encodes for the estrogen receptor and an estrogen-responsive reporter plasmid that in this case expresses luciferase. Since the HepG2 cells must be transfected for each experiment, we also transfected in a constituitively active β-galactosidase reporter plasmid that allowed us to monitor transfection efficiency (Figure 4). To determine the effect of estrogen receptor concentration on response, the cells were transfected with decreasing amounts of estrogen receptor plasmid and then treated with various doses of the two weakly estrogenic, hydroxylated polychlorinated biphenyls 2′,4′,6′-trichloro-4-biphenylol and 2′,3′,4′,5′-tetrachloro-4-biphenylol, either singly or in equimolar combination. As expected, peak activity declined with decreasing estrogen receptor concentration (Figure 5). In contrast to the results reported by Arnold et al. (1996), however, the combined activity of these two hydroxylated polychlorinated biphenyls was additive, not synergistic. No synergism was detected at any estrogen receptor concentration. Thus our results do not support the hypothesis that synergism occurs at low estrogen receptor concentrations.

MCF-7 Cells

The potential interactions of toxaphene and dieldrin were further investigated in MCF-7 human breast cancer cells, which were

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Estrogenic Activity (from page 3)

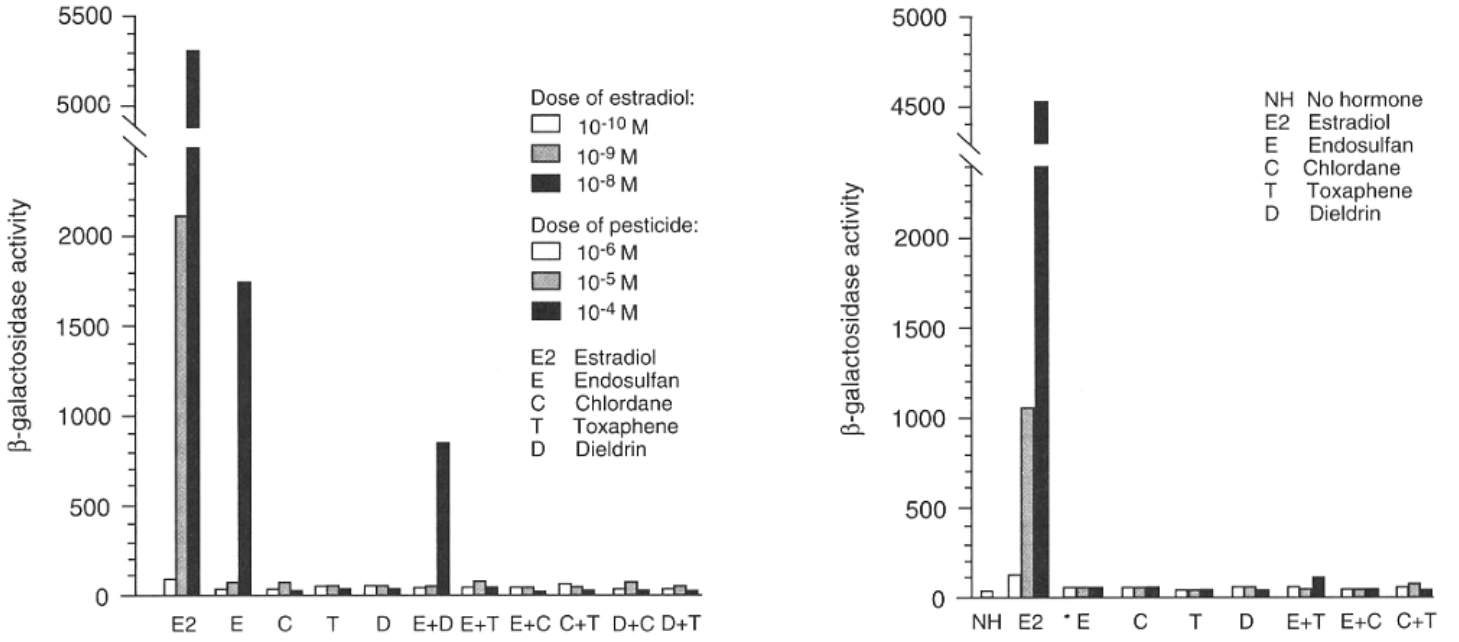

Figure 3
β-galactosidase activity induced by organochlorine pesticides using an estrogen-responsive reporter system in yeast expressing human estrogen receptor. The yeast strain BJ2407 was transformed with a yeast expression plasmid containing the human estrogen receptor and a reporter plasmid containing two copies of the vitellogenin estrogen-responsive element linked to the β-galactosidase gene. This strain was used to match experimental conditions reported by Arnold et al. (1996). The yeast was exposed to organochlorine pesticides alone or in combination for 24 hours and then assayed for β-galactosidase activity. (Left) Yeast cells were maintained in the presence of copper to induce a high concentration of estrogen receptor. (Right) Yeast cells were maintained in the absence of copper to induce low estrogen receptor concentrations. NH, no hormone; included as a negative control.

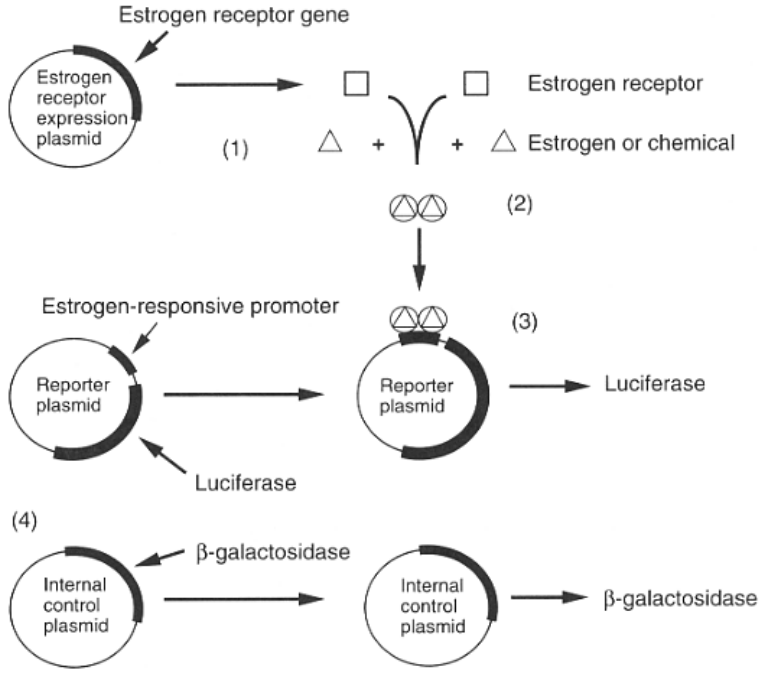

Figure 4
HepG2 steroid receptor assay. The estrogen receptor is expressed from its expression plasmid in an inactive form (1). Binding of estrogen or chemical ligand to the receptor results in dimerization and activation of the receptor to a form capable of binding to the estrogen-responsive element on the promoter of the reporter plasmid (2), resulting in production of the enzyme luciferase (3). Luciferase metabolizes luciferin in the presence of ATP, Mg^{2+}, and O_2 and emits light. The internal control plasmid expresses β-galactosidase (4). β-galactosidase activity is measured in a colorimetric assay.

derived from a patient with metastatic, infiltrating ductal carcinoma of the breast. MCF-7 cells contain endogenous estrogen receptors and proliferate in response to estrogen or estrogenic chemicals. This cell line is used by laboratories throughout the world to study estrogen receptor function. A number of laboratories have published results using MCF-7 cells to assess chemical interaction with the estrogen receptor (Soto et al., 1995; Villalobos et al., 1995; vom Saal et al., 1995; Reel et al., 1996), and the MCF-7 cell proliferation assay (also known as the E-screen) has been proposed for use in screening chemicals for estrogenic activity. Soto and coworkers (1994) previously reported that both toxaphene and dieldrin induced limited proliferation of MCF-7 cells only at the highest concentration (10^{-5} M).

In our study (Ramamoorthy et al., 1997a), 10^{-5} to 10^{-7} M concentrations of toxaphene, dieldrin, and dieldrin plus toxaphene (equimolar mixture) did not induce cell proliferation, whereas 10^{-9} M estrogen caused a 6-fold increase in cell growth (Figure 6). Similar results were observed in MCF-7 transient transfection studies using estrogen-responsive promoter reporter constructs (Ramamoorthy et al., 1997a).

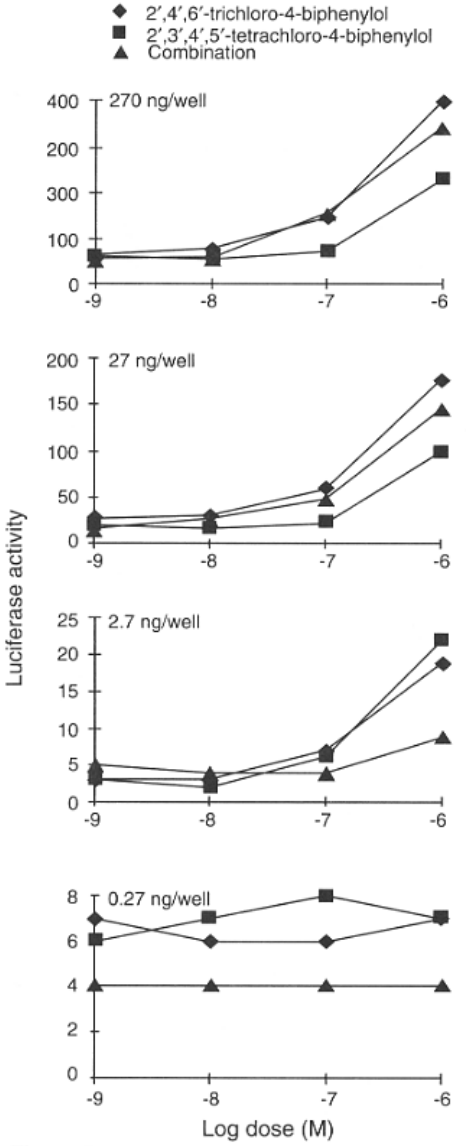

Figure 5
Estrogenic activity of hydroxylated polychlorinated biphenyls in HepG2 cells cotransfected with various concentrations of estrogen receptor. HepG2 cells were cotransfected with C3-LUC estrogen-responsive reporter plasmid (405 ng/well) and human estrogen receptor expression plasmid (270, 27, 2.7, or 0.27 ng/well). Cells were treated with different concentrations of 2′,4′,6′-trichloro-4-biphenylol and 2′,3′,4′,5′-tetrachloro-4-biphenylol, either singly or in combination. Luciferase activity was determined after treatment for 24 hours and is expressed as relative light units divided by β-galactosidase activity.

These results demonstrate minimal estrogenic activity and no apparent synergism of toxaphene and dieldrin in MCF-7 cells.

Competitive Binding Assay

Binding of organochlorine pesticides to the estrogen receptor was also determined in a competitive binding assay by incubating MCF-7 cells with 10^{-9} M [^{3}H]17β-estradiol in the presence or absence of 2×10^{-7} M unlabeled estrogen (to determine nonspecific binding), toxaphene (10^{-5} M), dieldrin (10^{-5} M), and equimolar concentrations of the dieldrin-toxaphene mixture (10^{-5} M). In a competitive binding assay, the estrogen receptor is labeled with radiolabeled estradiol. Compounds that bind to the estrogen receptor displace the radiolabeled estradiol, thereby causing a measurable decrease in receptor-associated radioactivity.

Our results (Figure 7) demonstrate that binding of [^{3}H]17β-estradiol to the estrogen receptor in MCF-7 cells was not competitively decreased by cotreatment with 10^{-5} M toxaphene, dieldrin, or the mixture of toxaphene and dieldrin, whereas a 200-fold excess of unlabeled estrogen caused a 94% decrease in estrogen receptor binding. These results indicate minimal competition by toxaphene, dieldrin, or dieldrin plus toxaphene for the human estrogen receptor in MCF-7 cells.

A competitive binding assay was also performed using estrogen receptor isolated from the mouse uterus. As illustrated in Figure 8, toxaphene, dieldrin, and a mixture containing equimolar concentrations of both compounds did not competitively displace [^{3}H]estrogen from the mouse uterine estrogen receptor. Our results are in direct contrast to the reported synergistic binding of these compounds using high concentrations of baculovirus-expressed human estrogen receptor (Arnold et al., 1996).

In Vivo Studies

Uterine weight increases in response to estrogenic stimulation. This response has been used for over 70 years to assess estrogenic activity of chemicals, and today it remains as one of the most useful short-term, in vivo assays for estrogenicity. The typical assay uses a female rat or mouse with low levels of endogenous estrogen (ovariectomized adults or immature animals). The animals are dosed daily with test chemical for 3 to 4 days. The animals are killed 24 hours after the last treatment, and the uteri are removed for weighing. Since uterine progesterone receptor concentration and peroxidase activity are gene-responsive markers, they also increase following estro-

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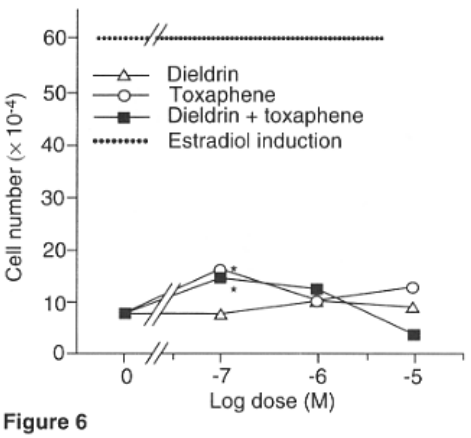

Figure 6
Effects of estrogen and organochlorine pesticides on proliferation of MCF-7 cells. The cells were treated with 10^{-9} M estrogen or 10^{-7}, 10^{-6}, or 10^{-5} M dieldrin, toxaphene, or a mixture of dieldrin and toxaphene for 11 days. The results are presented as means of three separate experiments. Significant induction of cell growth was observed only for 10^{-7} M toxaphene and the 10^{-7} M mixture of dieldrin and toxaphene; 10^{-9} M estrogen caused a greater than 6-fold induction in cell proliferation.

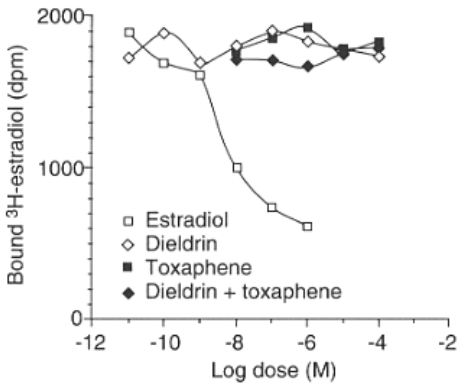

Figure 7
Competitive binding of estrogen, dieldrin, toxaphene, and dieldrin plus toxaphene to the estrogen receptor in MCF-7 cells. MCF-7 cells were treated for 2 hours with 10^{-9} M [^{3}H]estrogen in the presence or absence of various unlabeled competitors. Whole-cell extracts were isolated and treated with dextran-coated charcoal to remove unbound ligands, and estrogen receptor binding was determined.

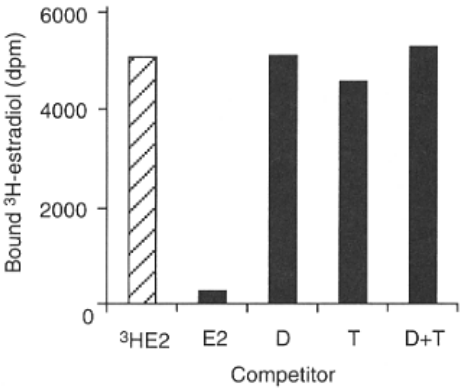

Figure 8
Competitive binding of organochlorine pesticides to the mouse uterine estrogen receptor. Uterine estrogen receptor was incubated with [^{3}H]estradiol alone (^{3}HE2) or in combination with unlabeled estrogen (E2, 2×10^{-7} M), dieldrin (D, 10^{-5} M), toxaphene (T, 10^{-5} M), and dieldrin plus toxaphene (D+T, 10^{-5} M); and the displacement of radiolabeled hormone was determined. Only unlabeled estrogen significantly decreased binding of [^{3}H]estrogen to the uterine estrogen receptor.

genic stimulation. With the use of receptor antagonists, these responses have been shown to be estrogen-receptor-mediated biological activities.

We assessed the activity of toxaphene and dieldrin using a standard immature mouse protocol for estrogenicity. Toxaphene and dieldrin exhibited minimal estrogenic activity for uterine weight increase or induction of progesterone receptor concentration or peroxidase activity, and no apparent synergistic interactions involving these in vivo estrogen responses were observed for the mixture of toxaphene and dieldrin (Figure 9).

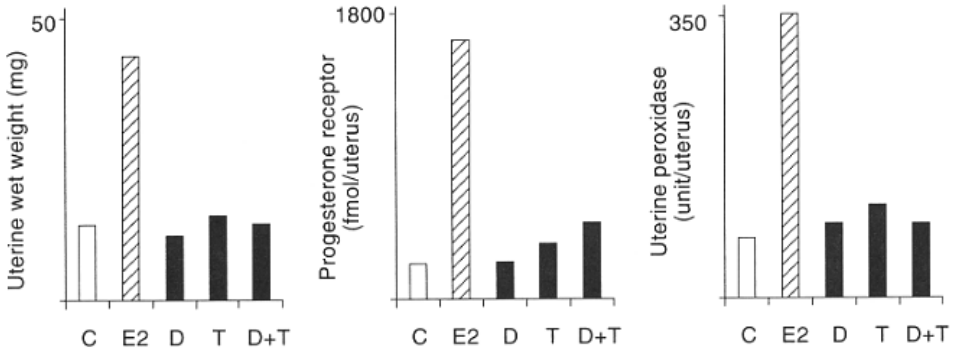

Figure 9
Effects of estrogen (E2), dieldrin (D), toxaphene (T), and dieldrin plus toxaphene (D+T) in the immature B3C3F1 female mouse uterus. Animals were treated with the various compounds for three days prior to termination. The doses were 0.02 µg/kg/day (estrogen) or 60 µmol/kg/day (all other groups). A vehicle control group (C) was also included. The uterine wet weight data are means from 6 to 9 uteri. Progesterone receptor binding and uterine peroxidase activity data are means from triplicate determinations with extracts from pooled uteri. An UPO activity unit is defined as the amount of enzyme required to produce an increase of 1 absorbance unit/minute.

Summary

Data obtained from four separate laboratories that used both in vivo and in vitro models for assessing estrogenic activity are combined in this article. No synergistic interactions were observed with combinations of weakly estrogenic chemicals using various estrogen receptor concentrations for nine different estrogen-dependent responses in the mouse uterus, MCF-7 human breast cancer cells, yeast-based assays, and HepG2 human hepatoma cells. The estrogenic activity of binary mixtures of the tested organochlorine pesticides was additive regardless of estrogen receptor concentration.

There were major differences in the results obtained in the yeast-based assay used in this study and the results previously reported by Arnold and coworkers (1996) despite the fact that both studies used the same yeast strain transformed with the human estrogen receptor and a comparable reporter system. However, the failure to observe synergistic interactions in the yeast-based assays used in the present study are also complemented by results showing that these chemical mixtures do not elicit synergistic estrogen-responsiveness in the female mouse uterus, MCF-7 human breast cancer cells, and HepG2 human hepatoma cells.

Thus synergism of chemical mixtures is not a universally observed response. Based on the results from our laboratories and others (Ashby et al., 1997; Ramamoorthy et al., 1997a; Ramamoorthy et al., 1997b; Arcari and Gierthy, 1997; Weise et al., 1997), there is insufficient evidence at the present time to incorporate synergism in the hazard assessment of weakly estrogenic chemical mixtures. Further analyses will be required to determine if synergism occurs in other estrogen-responsive systems. The scientific reasons for the results observed by Arnold and coworkers (1996) remain of interest and require further investigation.

Acknowledgments

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CIIT Hosts Career Panel

CIIT postdoctoral fellows learned about strategies for future employment and career opportunities during "Careers in Toxicology," an Institute-sponsored panel discussion held on February 20 at CIIT. Panel members were Dr. Hudson Bates, Manager of Regulatory Affairs, Nickel Producers Association; Dr. Margaret Lewandowski, Environmental Health and Safety Associate, BASF Corporation; Dr. Glenn Simon, Director of Toxicology, Rhone-Poulenc; Dr. Kathleen Sulik, Professor, Department of Cell Biology and Anatomy, University of North Carolina at Chapel Hill; and Dr. Rochelle Tyl, Director of Life Science and Toxicology, Research Triangle Institute.

Dr. Gaido

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Kevin W. Gaido received a B.S. degree in biochemistry from the University of Notre Dame in 1982 and a Ph.D. degree in pharmacology and toxicology from West Virginia University in 1986. He was a postdoctoral fellow at CIIT from 1986 to 1989 and joined the senior scientific staff in 1989. Dr. Gaido's research involves receptor-mediated mechanisms of toxicity.

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Kenneth S. Korach received a Ph.D. degree in endocrinology from the Medical College of Georgia in 1974. From 1973 to 1976, he was a postdoctoral biological chemistry research fellow at Harvard Medical School in the laboratory of the late Professor Lewis Engel. He also received a Ford Research Fellowship while at Harvard. Dr. Korach joined the National Institute of Environmental Health Sciences (NIEHS) in 1976. He has headed a research group investigating the basic mechanisms of estrogen hormone action in reproductive tract and bone tissues. Dr. Korach is currently Scientific Program Director of the Environmental Diseases and Medicine Program, Chief of the Laboratory of Reproductive and Developmental Toxicology, and Chief of the Receptor Biology Section at NIEHS.

Stephen H. Safe holds a D.Phil. degree in bioorganic chemistry from Oxford University, Great Britain. He was a research assistant in bioorganic chemistry at Oxford University and in biochemistry at Harvard University. He held a faculty position in the Department of Chemistry at the University of Guelph from 1973 to 1981. Dr. Safe is currently a distinguished professor in the Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station. He received a Burroughs Wellcome Toxicology Scholar Award, 1989 to 1994, and a Sigma Xi Distinguished Achievement Award in 1995.

Environmental Law Students Visit CIIT

A class of environmental law students from the University of Virginia visited CIIT on February 24 to learn about CIIT research and risk assessment. Instructor of the course is Richard A. Merrill, L.L.B., Daniel Caplin Professor of Law at the University of Virginia and General Counsel for CIIT. Speakers were Dr. James Bond, Dr. Paul Foster, Dr. Roger McClellan, and Dr. Michele Medinsky. (Left to right) Stephen Rubin, Justin Savage, Professor Merrill, Dr. McClellan, Ann Coyle, Erinn Kelly, Steve Nickelsburg, Laura Schuler, Pamela Bush, Eliza Platts-Mills, Valerie Hurt, Dr. Bond, Dr. Foster, and Dr. Medinsky.

Testimony on the Scientific Basis for Changes in the National Ambient Air Quality Standards for Ozone and Particulate Matter

Roger O. McClellan

The United States Environmental Protection Agency (U.S. EPA) has recently proposed major changes in the National Ambient Air Quality Standards (NAAQS) for ozone and particulate matter. Roger O. McClellan served as a member of both of the Clean Air Scientific Advisory Committee panels that reviewed the criteria documents and staff position papers on these two criteria pollutants. On February 5, 1997, Dr. McClellan testified on the scientific basis for the proposed changes at the request of the Subcommittee on Clean Air, Wetlands, Private Property and Nuclear Safety, Committee on Environment and Public Works, United States Senate. This report is a slightly abbreviated version of his written testimony. Some related material has been added to provide the reader a more complete picture of the activities related to the EPA proposal to change the NAAQS for both ozone and particulate matter.

Legislative Basis for National Ambient Air Quality Standards

The legislative basis for the Clean Air Act is well known to all of you. However, I would like to highlight several key points to provide a basis for my remarks. The Clean Air Act directs the Administrator of the United States Environmental Protection Agency (U.S. EPA) to identify pollutants that "may be reasonably anticipated to endanger public health and welfare" and to issue air quality criteria for them. These air quality criteria are intended to "accurately reflect the latest scientific knowledge useful in indicating the kind and extent of all identifiable effects on public health or welfare which may be expected from the presence of [a] pollutant in the ambient air...."

For these "criteria pollutants," the administrator is directed to propose and promulgate "primary" and "secondary" National Ambient Air Quality Standards (NAAQS). In the interest of brevity, I will consider only the primary standard-setting process in this testimony. The primary standard is defined in the Act as one "the attainment and maintenance of which, in the judgment of the Administrator, based on the criteria and allowing an *adequate margin of safety*, [is] requisite to protect the public health." The legislative history of the Clean Air Act indicates that the primary standard is to be set at "the maximum permissible ambient air level...which will protect the health of any *[sensitive] group* of the population" and that for this purpose "reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group." The standard is viewed as sufficient whenever there is "an absence of *adverse effects* on the health of a statistically related sample of persons in sensitive groups from exposure to ambient air."

The courts have held that the "*margin of safety*" requirement for primary standards was intended to address uncertainties associated with inconclusive scientific and technical information available at the time of standard setting. And further, it was intended to provide protection against hazards that research has not yet identified or whose medical significance is a matter of disagreement. In setting a margin of safety, the EPA considers such factors as the nature and severity of the health effects involved, the size of the sensitive population(s) at risk, and the kind and degrees of uncertainties that must be addressed. The margin of safety comes into play at the boundary between conclusive evidence of adverse effects related to pollutant exposure and levels of exposure where there is no conclusive evidence of adverse effects with unknown or only partially quantified risks. The selection of a particular approach to providing an adequate margin of safety has been viewed by the courts as a policy choice left specifically to the Administrator's judgment. The primary standard is to be set without regard to the cost of its implementation.

A section of the Clean Air Act enacted in 1977 requires that "not later than December 31, 1980, and at five-year intervals thereafter, the Administrator shall complete a thorough review of the criteria published under Section 108 of the National Ambient Air Quality Standards...and shall make such revisions in such criteria and standards and promulgate such new standards as may be appropriate...." The Act requires that an independent scientific review committee be appointed to "complete a review of the criteria...and the national primary and secondary ambient air quality standards...and shall recommend to the Administrator any new standards and revisions of existing criteria and standards as may be appropriate...." This function is carried out by the Clean Air Scientific Advisory Committee (CASAC) of the U.S. EPA Science Advisory Board.

Put in its simplest form, the Clean Air Act requires the Administrator to develop criteria and promulgate standards for certain air pollutants to protect against *adverse effects* in the public, including *sensitive populations*, with an *adequate margin of safety*. As clearly implied by the statutory language, levels of pollutant exposures can be identified that cause effects, while lower levels of exposure will be without effect (that is, a threshold for response). A "margin of safety" is then used to select a lower level for the standard, a level that, if attained, should not result in unacceptable risk.

Ozone Standard

The current primary NAAQS for ozone is set at 0.120 ppm with a one-hour averaging time. Attainment of the standard occurs when the expected number of days per calendar year with a maximum hourly average concentration greater than 0.120 is equal to or less than one. Operationally, the standard is exceeded if the 0.120 ppm hourly average concentration is exceeded a fourth time in a three-year period.

In 1993, the EPA Administrator reaffirmed the 0.120 ppm standard with a one-hour averaging time. At the same time, the Agency initiated the preparation of an updated criteria document on ozone and made plans for preparation of a Staff Paper for CASAC review of both the Criteria Document and Staff Paper (U.S. EPA, 1996a, 1996f). The CASAC came to closure on the Criteria Document on November 28, 1995, and on the Staff Paper on November 30, 1995.

The review process for the NAAQS for ozone considered a substantial amount of new data published since the last CASAC review was concluded in early 1989. The data came from four sources: controlled human exposure studies, field studies of children and healthy adults, analysis of air quality data and hospital admissions, and laboratory animal studies.

The controlled human exposure studies involved individuals engaged in light to heavy exercise with exposure to ozone over a range of concentrations for 1 to 6.6 hours. Decrements in pulmonary function and increases in symptoms of respiratory responses were dependent on exposure concentration and exposure duration. However, there was substantial intergroup variability in response as well interindividual variability for repeated exposures. The results of these studies support the use of an eight-hour averaging time.

The field studies of children in summer camp and exercising adults took advantage of naturally occurring variations in ambient ozone concentrations. Lung function tests were performed in all the individuals. A small but substantially significant association between ozone concentrations and reduced pulmonary function was observed for both groups. The relationship between increased ozone and decreased function was approximately linear with no clear threshold for an absence of effect.

The hospital admission studies examined the association between daily ozone concentrations and daily hospital admissions for respiratory effects. Asthmatics were identified as one susceptible subpopulation. Linear relationships were observed with increasing ozone and increased admissions with no clear evidence of a threshold.

The animals studied revealed effects that were qualitatively similar to those seen in people. The results of a key study with rats and mice exposed five days per week to ozone at exposure levels of 0.12 ppm and higher for two years suggested that long-term exposure at current ambient concentrations of ozone was unlikely to produce serious, irreversible changes in the lungs. I found those findings reassuring; they reduced my concern for the long-term impact from brief exposures that produce reversible effects. Based on consideration of all of the data, the EPA staff paper recommended consideration of an eight-hour averaging time standard in the range of 0.070 to 0.090 ppm and a potential for multiple exceedances.

The CASAC provided review comments and closure letters on both the Ozone Criteria Document (Wolff, 1995a) and the Ozone Staff Position Paper (Wolff, 1995b). Based on the information presented in the Ozone Criteria Document and analyzed in the Ozone Staff Paper, the CASAC reached several key conclusions:

1. Ozone remains an appropriate indicator for use as an indicator of photochemical oxidants.
2. An eight-hour averaging time standard was more appropriate for a human health-based standard than a one-hour averaging time.
3. "The weight of the evidence indicates that there is no threshold concentration for the onset of biological responses due to exposure above background concentrations" and thus "there is no 'bright line' which distinguishes any of the proposed standards (either the level or the number of allowable exceedances) as being significantly more protective of public health."
4. The CASAC Ozone Panel members expressed a range of preferences for the level of the standard.

No. of panel members	Preferred ozone level (ppm)
1	0.090–0.100
3	0.090
1	0.080–0.090
3	0.080
2	Policy call

In December 1996, the U.S. EPA (1996d) published the proposed rule for a new NAAQS for ozone. The EPA proposed setting the primary NAAQS at 0.080 ppm with an eight-hour averaging time. The primary standard would be met at an ambient air quality monitoring station when the three-year average of the annual third-highest, daily maximum, eight-hour average ozone concentration is less than or equal to 0.080 ppm. The EPA also solicited comments on an alternative eight-hour level of 0.090 ppm and 0.070 ppm as well as on retaining the current primary standard.

It is my professional judgment, as I noted during the CASAC deliberations, that the primary ozone standard should be set at 0.090 ppm with an eight-hour averaging time and the use of the three-year average of the annual third-highest maximum of eight-hour average ozone concentration to evaluate attainment of the standard. I would personally prefer to have some form of averaging of data from multiple monitoring sites when available rather than using the highest monitor to determine attainment of the standard. The use of multiple monitors would better reflect population exposure and aggregate public health risk.

My professional opinion on the level and form of the ozone standard was shaped by consideration of data such as those shown in Table 1. The table is adapted from material provided in the CASAC closure letter (Wolff, 1995b). This table is based on a study by Thurston et al. (1992), who examined the relationship between ozone levels and hospital admissions. The model assumed ozone effects down to a background level of 0.040 ppm. The first row in the table (excess admissions) was prepared by EPA staff and included in the draft Ozone Staff Paper. It may be noted that the excess admissions for various ozone control scenarios included 210 cases for the present standard to a range of 60 to 240 cases for alternative standards. For comparison, the present situation (as is) is estimated to result in about 400 cases. The five lower rows in the table were prepared by CASAC Panel members. The second row, which reports the excess admissions as a percentage change from the present standard, at first glance appears to suggest considerable difference among the several options. However, the other rows are worthy of detailed consideration before a final conclusion is drawn.

The third row includes both the excess admissions due to ozone-aggravated asthma above the level of the standard and those cases related to ozone below the level of the standard down to background. The relative effect of the different options now appears to be much less, as seen from examining row 4. Let us now turn our attention to row five (all asthma admissions), with a baseline of approximately 30,000 cases. When this value is compared with that for the various options, ozone-aggravated asthma admissions clearly represent only a small fraction of the total number of cases, and the difference in impact of the various options for the ozone standard is small.

It is especially important to note that 680 asthma admissions per year are attributed to background levels of ozone, which is assumed to be 0.040 ppm of ozone. These calculated cases are a reflection of the linear exposure-response models used to calculate the ozone-attributable cases.

The primary public health issue relates to the approximately 30,000 cases of asthma admissions. I can personally identify with these cases since one of my children, who grew up in the clean air of New Mexico, was and is an asthmatic. My first-hand recollection of his suffering from asthma attacks triggered by multiple causes such as animal dander, grass pollens, extreme cold air, and heavy exercise left an imprint on me. As much as anyone, I would like to better understand what causes asthma, including the vexing issue of why asthma rates are increasing, especially when air quality is improving. I have serious reservations about the extent to which ozone exposures are a significant contributor to the asthma problem.

Let me hasten to add that the health impacts of ozone are not restricted to effects in asthmatics. However, Table 1 clearly illustrates the importance of considering the estimated impacts of pollutant exposures within the broader context of other risk factors for specific health outcomes. In my opinion, the ultimate concern of society is for the aggregate risks from all causes and how best to achieve an overall reduction.

I am personally a strong advocate of comparative risk analyses such as detailed above to help guide decisions on important

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societal issues. It is my understanding that the EPA Administrator can use analyses such as this in making decisions on the ozone standard although the Administrator is prohibited from explicitly considering costs of implementing the standard.

Before leaving the ozone issue, let me note that I believe it to be unfortunate that the Clean Air Act prohibits the consideration of cost in setting the standard. In my opinion, the best interests of society would be served if attention could be focused on the best buy for societal actions that will reduce health risks, including those of ozone. Further reductions in ozone may not be cost-effective relative to other options for reducing risks and improving health.

The explicit consideration of the cost of achieving the various options would be of substantial value in making a decision that is likely to have a multibillion-dollar impact on society.

Particulate Matter

The current particulate matter (PM) standard was promulgated in 1987 when the indicator for particles was changed from total suspended particulates (TSP) to PM_{10}, the latter referring to particles sampled in a manner that 50% of the particles with an aerodynamic diameter of 10 μm will be collected. As particle size decreases, a greater portion of the particles are collected with essentially all particles smaller than about 7 μm collected with 100% efficiency. The 24-hour PM_{10} standard was set at 150 $\mu g/m^3$, with no more than one expected exceedance per year; and the annual PM_{10} standard was set at 50 $\mu g/m^3$, expected arithmetic mean. The PM_{10} standard is thought to provide a more health-protection-relevant metric for controlling exposure than the old TSP metric.

The NAAQS for particulate matter is not chemical-specific, unlike the chemical-specific standards for other criteria pollutants and most other substances regulated by the EPA. The particulate matter standard applies to a broad class of chemically and physically diverse substances that exist as discrete particles (liquid droplets or solids) over a wide range of sizes. Particulate matter is characterized as to its mass within a given size range.

Knowledge of the size and origin of particles is fundamental to understanding their potential health effects and, ultimately, to the establishment of appropriate standards and control strategies (Figure 1). Particles in the atmosphere vary widely as to their size and origin. The smallest, or ultrafine, particles arise from condensation of vapor and a clustering of individual molecules. The ultrafine mode of particles is not shown in Figure 1 because they are generally smaller than 0.1 μm in size and contribute little mass compared to the fine and coarse modes of particles. The ultrafine particles grow in size and coagulate in the atmosphere to form fine (or accumulation mode) particles that are typically less than a micrometer in diameter. Other larger or coarse particles typically arise by mechanical processes such as the erosion of soil.

The size of particles influences the dynamics of particles in the atmosphere. The finest particles coagulate to become larger particles. These particles may be removed from the atmosphere by rain. The largest particles may settle out due to gravity. Small and medium particles may be transported long distances by the wind. As a former resident of Albuquerque, New Mexico, I can recall that in the spring we sometimes had some Arizona dust blow through when the winds were from the west and Texas and Oklahoma dust blow through when the winds were from the east. I am sure that it included PM_{10} particles and, indeed, some $PM_{2.5}$ particles.

Scientists studying particles in the atmosphere have appreciated the need to better understand particle size, and this has led to the development of methods for collecting particles and characterizing the particles as to size. Just as size influences how particles behave in the atmosphere, size also influences their potential for being inhaled and deposited in the respiratory tract and for causing adverse health effects. The concern for how particles of different sizes could affect health also influenced the design of air sampling devices.

Some of the conventions for characterizing particles as to their size are illustrated in Figure 1. In particular, note the size fractions designated as (1) total suspended particulates (TSP); (2) particulate matter,

Table 1
Estimated Hospital Admissions for Asthmatics in the New York City Area for Various Ozone Control Scenarios

Row	Hospital admissions	1H1EX* 0.120	1H1EX 0.100	8H1EX 0.100	8H1EX 0.090	8H1EX 0.080	8H1EX 0.070	8H5EX 0.090	8H5EX 0.080	As is
1	Excess admissions[a]	210	130	240	180	110	60	180	120	≡ 385[d]
2	% Δ from present standard	0%	-38%	+14%	-14%	-48%	-71%	-14%	-42%	+83%
3	Excess + background[b]	890	810	920	860	790	740	860	800	1065[e]
4	% Δ from present standard	0%	-9%	+3%	-3%	-11%	-17%	-3%	-10%	+20%
5	All asthma admissions[c]	28,295	28,215	28,325	28,265	28,195	28,145	28,265	28,205	28,470[f]
6	% Δ from present standard	0%	-0.3%	+0.1%	-0.1%	-0.4%	-0.5%	-0.1%	-0.3%	+0.6%

*1H1EX: 1 hour averaging time, 1 exceedance, and concentration of 0.120.
[a]Excess asthma admissions attributed to ozone levels exceeding a background concentration of 0.04 ppm. From Table VI-2, page 155 in the August 1995 OAQPS Draft Staff Paper.
[b]Asthma admissions included in (a) plus those due to background ozone concentrations. Admissions due to background = 1065[e] - 385[d] = 680.
[c]Asthma admissions due to all causes = 28,470[f] - 385[d] + excess admissions from row 1.
[d]Estimated from Figure V-15, page 125 in the August 1995 OAQPS Draft Staff Paper.
[e]From page 127, line 13 in the August 1995 OAQPS Draft Staff Paper.
[f]Total admissions from asthma = total asthmatics (365,000; from page 126, line 24) × hospitalization rate (78/1000 asthmatics; from page 126, line 29).
Adapted by the Clean Air Scientific Advisory Committee Ozone Panel from the EPA Ozone Staff Paper (Wolff, 1995b). The notations in the footnote above refer to the August 1995 OAQPS Draft Ozone Staff Paper (U.S. EPA, 1996f).

10 micron size (PM_{10}); and (3) particulate matter, 2.5 micron size ($PM_{2.5}$).

The TSP sample represents essentially all the particles that can be drawn into a high-volume sampler. This includes many large, heavy particles that have a very low probability of being inhaled and reaching the lungs. These particles are clearly a nuisance but are not of major health concern.

Recognition that smaller particles can be inhaled led to the development of methods for collecting smaller particles, including the PM_{10} fraction. As an aside, it should be noted that some of the smallest of the coarse-mode particles are collected in the $PM_{2.5}$ sample. These are collected with devices that collect 50% of the particles 10 μm in aerodynamic diameter. Particles larger than 10 μm are collected less efficiently, while smaller particles are collected more efficiently. The $PM_{2.5}$ fraction is similar except that the cut-off is set at 2.5 μm.

In 1979 and 1980, EPA was struggling with the issue of developing a size-selective NAAQS for particulate matter to replace the TSP standard set in 1971. Several different size cuts were under consideration, and there was a flurry of activity to gather field data using new devices, including some calibrated for PM_{15}, PM_{10}, and $PM_{2.5}$. However, the debate was largely removed from EPA's regulatory agency in 1981 when the International Standards Organization adopted a 10-μm cut point for particles that could penetrate to the human thorax (that is, the trachea and conducting and pulmonary airways). This focused attention on a PM_{10} standard that was formally promulgated in 1987. With promulgation of the new standard and the need to demonstrate regulatory compliance, there was a general shift to PM_{10} measurements. TSP measurements were discontinued and, unfortunately, so were most measurements of $PM_{2.5}$. I have termed this phenomenon "looking under the regulatory lamppost" (McClellan, 1996). In general, after closure on the Particulate Matter Criteria Document and Staff Paper in 1986, the level of financial support for research on particulate matter dwindled.

In my opinion, the Agency took appropriate action to move to a PM_{10} indicator in 1987. The use of the PM_{10} indicator has been effective in guiding actions to control particulate air pollution and minimize the likelihood of adverse health effects attributable to particulate air pollution. From 1988 to 1995, there was a 22% reduction in the annual mean PM_{10} concentrations (see the U.S. EPA National Air Quality and Emissions Trends Report, 1995) (U.S. EPA, 1996c). This and a companion document (National Air Pollutant Emission Trends, 1990–1994) (U.S. EPA, 1995) are excellent references for gaining an appreciation of the substantial progress being made in improving air quality in the United States. Unfortunately, detailed data are not available on trends in $PM_{2.5}$ and $PM_{1.0}$ measurements. However, I suspect that substantial reductions have also occurred in the concentrations of these smaller particles.

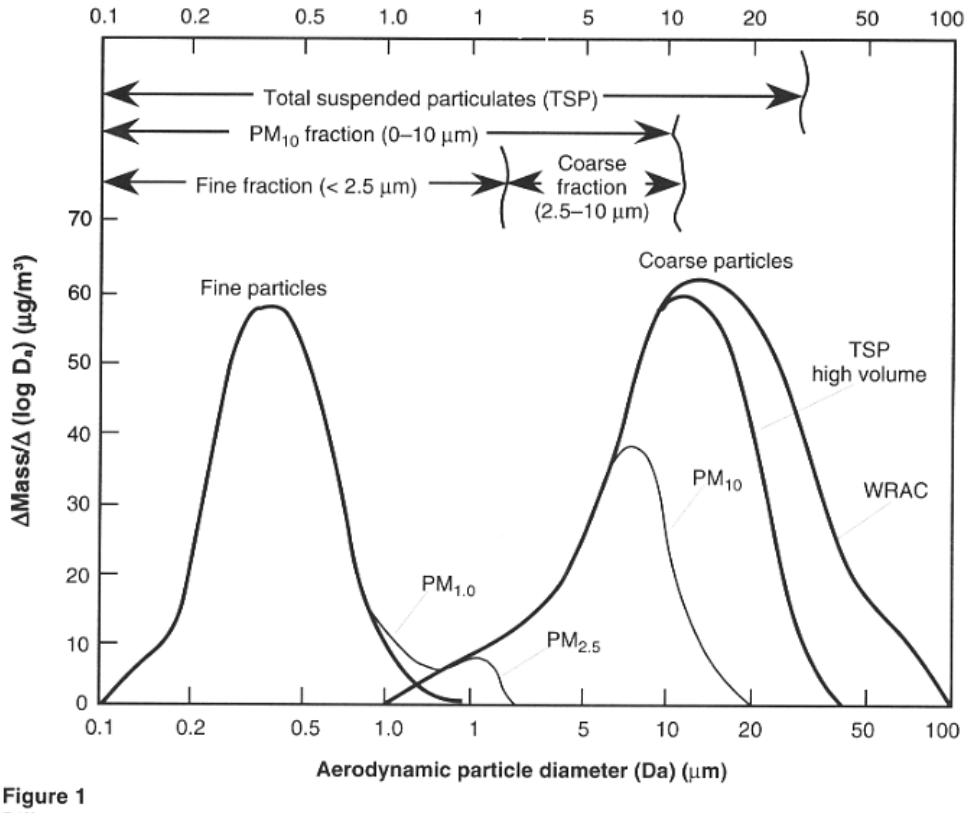

Figure 1
Different parameters used to describe the size distribution of airborne particulate matter (PM). WRAC, wide-range aerosol collector. (From Wilson and Suh, 1997.)

During the early 1990s, reports began to appear in the literature of time series analyses of particulate matter measurements and daily mortality. These were retrospective, opportunistic studies of data collected for other purposes. These studies frequently used techniques developed originally for econometric analyses. The techniques attempted to account for or filter out effects such as season of year and temperature that could influence mortality with the remaining statistical relationship between daily particulate matter and daily mortality quantified. Later studies attempted to take account of the role of other pollutants such as ozone and acid sulfates. A major handicap to the conduct of many of these studies was the lack of PM_{10} data. In many cases, the best available data were for TSP. These were then converted or extrapolated to PM_{10} values or, in some cases, even extrapolated to $PM_{2.5}$ values. On average, the investigators found about a 4% increase in daily mortality for a 50-μg/m^3 increase in PM_{10} concentration or extrapolated PM_{10} values.

Unfortunately, only a very few long-term prospective studies of cohorts of individuals have been conducted with associated measurements of particulate matter and other pollutants. Only rarely have long-term, multiyear studies been conducted with research-quality air pollution measurements made rather than depending on regulatory compliance measurements. The result is excessive dependence on the old TSP measurements or, more recently, PM_{10} measurements. Only very limited research has been done when both PM_{10} and $PM_{2.5}$ have been measured, and only very recently have some PM_{10} measurements been obtained. In the cohort studies, mortality rates after adjustment for smoking and other confounding variables have been related to the PM_{10} or $PM_{2.5}$ measurements or extrapolated values. EPA used the mortality estimates from two such prospective studies to conclude that there are premature deaths due to chronic exposure to PM.

In my opinion, the EPA staff and consulting scientists assisting the Agency did an admirable job of compiling all that is currently known about the health effects of particulate matter. Unfortunately, the price must now be paid for inadequate support of research on the effects of air pollution. In my professional judgment, the data base available today is not sufficient to establish a new particulate matter indicator or to select the level and form of a new standard.

The data suggest that high levels of particulate matter as experienced in the past are associated with increased morbidity and mortality. However, I must note that some investigators have suggested that the effect

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Testimony (from page 11)

measured is a general air pollution effect with particulate matter measurements serving as a surrogate measure of air pollution rather than as a causative agent. The data are reasonably strong for PM_{10}. Unfortunately, the dearth of $PM_{2.5}$ measurements serves as a serious obstacle to rigorously evaluating the association between $PM_{2.5}$ and multiple measures of health for specific populations, including those that might be especially susceptible. And we have no evaluations of possible association health indices and other particulate matter metrics such as $PM_{1.0}$ (that would more accurately reflect particles that have been recently formed) or particle size and chemical-specific metrics traceable to specific types of sources.

An absence of data on other plausible alternatives and the bright light of the regulatory lamp post keep drawing us back to evaluating associations with PM_{10} and, to a lesser extent, with $PM_{2.5}$ (McClellan, 1996). It has been argued that the only way to get funding for more $PM_{2.5}$ measurements is to get a $PM_{2.5}$ standard. Thus we are faced with the perverse situation of creating a standard to get scientific data rather than having a standard developed based on solid scientific data. Limited data recently obtained on PM_{10}, $PM_{2.5}$, and $PM_{1.0}$ size fractions suggest that EPA may be making a serious error in proposing a $PM_{2.5}$ standard to control health risks related to fine particles. In the western United States, where $PM_{2.5}$ measurements include substantial soil dust, the use of a $PM_{2.5}$ indicator may lead to exaggerated estimates of risk. These data strongly suggest that a $PM_{1.0}$ indicator may be more appropriate than the use of a $PM_{2.5}$ indicator.

The CASAC reviewed both the Criteria Document (U.S. EPA, 1996b) and Staff Position Paper (U.S. EPA, 1996g) on particulate matter. The CASAC issued closure letters to Administrator Browner on both documents (Wolff, 1996a, 1996f). Because of the high degree of uncertainty associated with scientific data on $PM_{2.5}$, the individual CASAC Panel members expressed a wide range of preferences with regard to setting $PM_{2.5}$ standards.

In December 1996, the EPA, acting under a court order, proposed new $PM_{2.5}$ standards to complement continuation of the existing PM_{10} standards (U.S. EPA, 1996e). The Administrator has proposed an annual average $PM_{2.5}$ standard of 15 $\mu g/m^3$ and a 24-hour average $PM_{2.5}$ standard of 50 $\mu g/m^3$. The proposed new annual $PM_{2.5}$ standard would be met when the three-year average of the annual arithmetic mean $PM_{2.5}$ concentrations, spatially averaged across an area, is less than or equal to 15 $\mu g/m^3$. The proposed new 24-hour $PM_{2.5}$ standard would be met when the three-year average of the 98th percentile of 24-hour $PM_{2.5}$ concentrations at each monitor within an area is less than or equal to 50 $\mu g/m^3$.

The EPA solicited comments on two alternatives. One alternative was for a highly precautionary response with an annual $PM_{2.5}$ standard down to 12 $\mu g/m^3$ and a 24-hour average $PM_{2.5}$ standard in the range of 20 to 50 $\mu g/m^3$. The second alternative was for a limited response with an annual $PM_{2.5}$ standard set at 20 $\mu g/m^3$ and a 24-hour average $PM_{2.5}$ standard set at up to 65 $\mu g/m^3$.

The serious shortcomings in the scientific data on $PM_{2.5}$ and on $PM_{1.0}$ led me to not support the promulgation of either an annual or a 24-hour $PM_{2.5}$ standard. I reluctantly noted that if EPA were going to propose a $PM_{2.5}$ standard, I would set the 24-hour standard at 75 $\mu g/m^3$ and an annual standard at 25 $\mu g/m^3$. These would represent levels that would likely not result in misdirected control strategies while $PM_{2.5}$, and hopefully other particulate matter metrics as well, are measured throughout the country. A national strategy to better characterize particulate matter air quality would also provide the groundwork for development of a cost-effective particulate matter control strategy. And, most importantly, there is an urgent need to initiate multiple long-term prospective epidemiologic studies to assess whether there is currently a particulate matter problem and, if so, what specific size or chemical fractions are responsible. There is an urgent need for research to establish a mechanism-based causal linkage between particulate matter fractions to be regulated and human disease.

To address research needs such as those I have outlined in general terms will require expenditures on the order of $50 million per year for five years compared to the less than $20 million EPA is expending on particulate matter research in 1997. The alternative to making the research investments and acquiring information for a science-based standard is to proceed blindly with development of standards that will have a multibillion-dollar impact and may or may not have a positive impact on human health. I urge Congress to provide EPA guidance for immediately initiating the expanded research program needed to establish science-based NAAQS for particulate matter.

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Wolff, G. T. (1996a). Closure Letter on Draft Air Quality Criteria for Particulate Matter from Chairman of Clean Air Scientific Advisory Committee to EPA Administrator. March 15, 1996.

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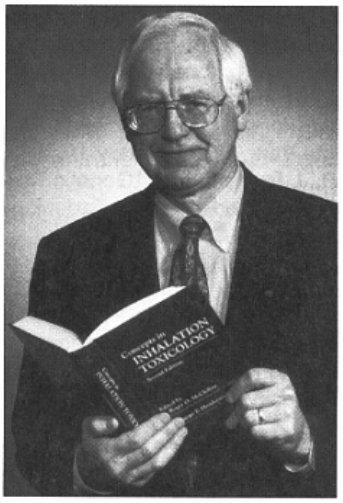
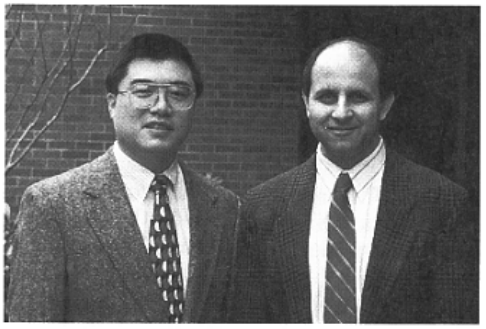


The Author

Roger O. McClellan serves as President of the Chemical Industry Institute of Toxicology (CIIT), a position he has held since September 1988. Prior to his appointment as CIIT President, Dr. McClellan was Director of the Inhalation Toxicology Research Institute and President and Chief Executive Officer of the Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico. During his 22 years with the Lovelace organization, he provided leadership in developing one of the world's leading research programs on the toxic effects of airborne materials.

Dr. McClellan has served in an advisory role to numerous public and private organizations. He has a long-standing interest in environmental and occupational health issues, especially those involving risk assessment and air pollution. He is a strong advocate of the need to integrate data from epidemiological, controlled clinical, laboratory animal, and cell studies to assess human health risks from exposure to toxic materials.

Dr. McClellan's advisory appointments have included service as Chairman of the United States Environmental Protection Agency (U.S. EPA) Clean Air Scientific Advisory Committee (CASAC) from 1988 through 1992. During this period, CASAC reviewed the Agency's criteria documents and staff papers on the National Ambient Air Quality Standards (NAAQS) for ozone, a review that concluded with EPA's reaffirmation of the standard of 0.120 ppm O_3, one-hour averaging time. Earlier, he served on the CASAC panel that reviewed the criteria documents and staff papers related to the NAAQS for particulate matter. This review was associated with the change from a total suspended particulates indicator to a particulate matter$_{10\ micron}$ (PM_{10}) indicator. Most recently, he has served on the CASAC panels reviewing scientific documentation that provides the basis for the NAAQS for both ozone and particulate matter.



Dr. Lei Zhang (left) with his mentor at CIIT, Dr. Bahman Asgharian.

Lei Zhang Accepts Position at Argonne National Laboratory

Lei Zhang has completed his postdoctoral training at CIIT and has accepted a position in aerosol research at Argonne National Laboratory, Argonne, Illinois.

Dr. Zhang received a Ph.D. degree in mechanical engineering from the State University of New York at Buffalo in 1994. His Ph.D. research involved the development of mathematical models to predict aerosol deposition in laboratory animals and humans. He also holds an M.S. degree in mechanical engineering from SUNY at Buffalo and a B.S. degree in mechanical engineering from Shanghai Jiao Tong University, Shanghai, China.

Dr. Zhang came to CIIT in August 1994 to work with Dr. Bahman Asgharian and others in advancing computer and mathematical airflow models for use in respiratory toxicology. He was involved in modeling particle and fiber deposition in the respiratory tract and performed experiments to determine the inhalability and deposition of particles in nasal airways of rodents. Dr. Zhang has published three articles on his research at CIIT.

Staff Publications

Bond, J. A., Himmelstein, M. W., Turner, M. J., and Medinsky, M. A. (1997). In vitro and in vivo approaches for extrapolation of 1,3-butadiene metabolism in laboratory animals to humans. In *Correlations Between In Vitro and In Vivo Investigations in Inhalation Toxicology* (Dungworth, D. L., Adler, K. B., Harris, C. C., and Plopper, C. G., editors). ILSI Press, Washington, DC, pp. 298–310.

Corton, J. C., Bocos, C.,* Moreno, E. S., Merritt, A., Marsman, D. S., Sausen, P. J., Cattley, R. C., and Gustafsson, J.-Å. (1996). Rat 17β-hydroxysteroid dehydrogenase type IV is a novel peroxisome proliferator-inducible gene. *Mol. Pharmacol.* 50, 1157–1166.

Dorman, D. C. (1997). Toxicosis in dogs and cats: Part 1. *Vet. Med.* 92, 139. [Editor's introduction to symposium.]

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Talcott, P. A.* and Dorman, D. C. (1997). Pesticide exposures in companion animals. *Vet. Med.* 92, 167–181.

Terry, K. K., Stedman, D. B., Bolon, B., and Welsch, F. (1996). Effects of 2-methoxyethanol on mouse neurulation. *Teratology* 54, 219–229.

Wolf, D. C. and Butterworth, B. E. (1997). Risk assessment of inhaled chloroform based on its mode of action. *Toxicol. Pathol.* 25, 49–52.

Wolf, D. C. and Hard, G. C.* (1996). Pathology of the kidneys. In *Pathobiology of the Aging Mouse* (Mohr, U., Dungworth, D. L., Ward, J., Capen, C. C., Carlton, W. W., and Sundberg, J., editors). ILSI Press, Washington, DC, pp. 331–344.

Zhang, L., Asgharian, B., and Anjilvel, S.* (1997). Inertial deposition of particles in the human upper airway bifurcations. *Aerosol Sci. Technol.* 26, 97–110.

*Non-CIIT author.

Staff Presentations and Interactions

Bond, J. A. Presentation, "Replacing Default Options with Scientific Knowledge: 1,3-Butadiene as a Case Study," University of Virginia environmental law class, CIIT, Research Triangle Park, NC, February 24.

Butterworth, B. E. Presided as President, meeting of the Board of Directors, Genotoxicity and Environmental Mutagen Society, Research Triangle Park, NC, February 13.

Casanova, M. Participated as Past President, Executive Committee meeting, Triangle Chapter of the Association for Women in Science, Research Triangle Park, NC, February 19.

Cattley, R. C. Presented three lectures and one laboratory, "Pathology of Liver, Pancreas, and Peritoneum," systemic pathology course, North Carolina State University, Raleigh, NC, February 7–13.

Cook, J. L. Instructor, Occupational Health and Safety Winter Institute, North Carolina Occupational Safety and Health Educational Resource Center, St. Petersburg, FL, January 28–31.

Dorman, D. C. Participated as member, workshop on Persian Gulf Illness, Military Working Dogs as Sentinels; U. S. Department of Defense, Armed Forces Institute of Pathology, Lackland Air Force Base, San Antonio, TX, January 21–22.

Fennell, T. R. Presentation, "Interspecies Extrapolation of Ethylene Oxide," Winter Toxicology Forum, Washington, DC, February 27.

Foster, P. M. D. Invited seminar, "Do Endocrine Disruptors Pose a Significant Risk to Human Reproduction?", Toxicology Program, North Carolina State University, Raleigh, NC, February 4.

Presentation, "You're Not Half the Man Your Father Was: Do Endocrine Disruptors Pose a Significant Threat to Human Reproductive Health?," Institute of Wildlife and Environmental Toxicology, Clemson University, Pendleton, SC, February 11.

Participated as member, meeting of the Reproductive and Developmental Effects Subcommittee, American Industrial Health Council, Washington, DC, February 12.

Presentation, "Environmental Endocrine Modulators: Is There a Human Reproductive Risk?", University of Virginia environmental law class, CIIT, Research Triangle Park, NC, February 24.

Gaido, K. W. Presentation, "In Vitro Assays for Detection of Chemical Interactions with Steroid Receptors," 22nd Annual Winter Toxicology Forum, Washington, DC, February 24.

Goldsworthy, T. L. Appointed to Organizing Committee for Annual International Conference on Carcinogenesis and Risk Assessment (Austin, TX, December 4–6, 1997), February 3.

Presided as President over annual meeting, North Carolina Society of Toxicology, Research Triangle Park, NC, February 8.

Kedderis, G. L. Participated as member, meeting of the Chemical Substances Threshold Limit Value (TLV) Committee, American Conference of Governmental Industrial Hygienists, with the German MAK Commission, Munich, Germany, February 22–24.

Seminar presentation, "Isolated Hepatocytes as In Vitro Models for the Biotransformation and Toxicity of Chemicals In Vivo," GSF Institute for Toxicology, Neuherberg, Germany, February 26.

Kuyper, B. J. Invited presentation, "Designing and Writing a Scientific Poster," Triangle Chapter of the Association for Women in Science, National Institute of Environmental Health Sciences, Research Triangle Park, NC, February 19.

McClellan, R. O. Invited testimony on National Ambient Air Quality Standards for Ozone and Particulate Matter, U.S. Senate Subcommittee on Clean Air, Wetlands, Private Property and Nuclear Safety, Committee on Environment and Public Works, Washington, DC, February 5.

Presentation, "How Adequate is the Scientific Basis for Changing the Particulate Matter Standard?"; Air Pollution Policy: A Symposium on the Proposed Standards for Ozone and Particulates, Resources for the Future, Washington, DC, February 10.

Participated as member, Institute of Medicine Committee on Environmental Justice, Washington, DC, February 10–11.

Participated as member, meeting of the Science Advisory Board Environmental Health Committee to review Proposed Cancer Risk Assessment Guidelines, U.S. Environmental Protection Agency, Washington, DC, February 13–14.

Invited lecture, "Mechanisms of Lung Toxicity"; panel member, "Assessing Health Risks of Airborne Toxicants"; course on Basic Mechanisms of Toxicology and Their Application and Risk Assessment, University of Sao Paolo and the Brazilian Society of Toxicology, Sao Paolo, Brazil, February 17–21.

Presentation, "Overview of CIIT," University of Virginia environmental law class, CIIT, Research Triangle Park, NC, February 24.

Invited presentation, "How Solid is the Scientific Basis of the NAAQS for Particulate Matter?", 22nd Annual Winter Toxicology Forum, Washington, DC, February 25.

Medinsky, M. A. Presentation, "Hazard Identification: How Do We Determine If A Chemical is Toxic?", University of Virginia environmental law class, CIIT, Research Triangle Park, February 24.

Participated as member, National Institutes of Health, Toxicology Study Section, Division of Research Grants, Washington, DC, February 26–28.

Miller, F. J. Presentation, "The Scientific Basis for EPA's Proposed Regulations for Particulate Matter and Ozone," American Enterprise Institute for Public Policy Research Conference on Clearing the Air: An Examination of EPA's Proposed Regulations for Particulate Matter and Ozone, Wohlstetter Conference Center, Washington, DC, February 10.

Mugford, C. A. Mentor, National Science Foundation Science-by-Mail program for elementary students, Museum of Sciences, Boston, MA, February 1.

Participated as member, meeting of the Membership Committee, Triangle Chapter of the Association for Women in Science, Research Triangle Park, NC, February 19.

Preston, R. J. Presentation, "Genetic Toxicology of Ethylene Oxide," Winter Toxicology Forum, Washington, DC, February 27.

Wong, B. A. Presented tutorial, "Aerosol Sizing Methods, review course for the U.S. Food and Drug Administration on Pharmaceutical Aerosols and Inhalation Toxicology; cosponsored by American Association for Aerosol Research, Rockville, MD, February 27.

North Carolina SOT Meets

In his new role as President, Dr. Thomas L. Goldsworthy presided over the Annual Meeting of the North Carolina Chapter of the Society of Toxicology, which was held in Research Triangle Park on February 8. Dr. Kevin Gaido is Secretary-Treasurer of the chapter and Dr. Susan Borghoff is a Councilor. CIIT scientists presented a total of 27 posters at the meeting.

Ambroso, J. L., Stedman, D. B., Terry, K. K., Elswick, B. A., and Welsch, F. The Amino Acid L-Serine Protects Against 2-Methoxyethanol-Induced Neural Tube Defects in CD-1 Mice.

Babaï, D. H., Schlosser, P. M., Portier, C. J.,* and Gaido, K. W. Mathematical Analysis of Variability in the Yeast Assay for Estrogen Activity.

Bergeron, R. M. and Gaido, K. W. Bisphenol A and 17β-Estradiol Differentially Regulate Cellular Proliferation and Estrogen-Responsive Gene Expression.

Butterworth, B. E., Templin, M. V., Constan, A. A., Wolf, D. C., and Wong B. A. Regenerative Cell Proliferation Correlates with Eventual Tumor Formation in the BDF1 Mouse.

Christensen, J. G., Gonzales, A. J., and Goldsworthy, T. L. Effects of Hepatic Tumor Promoters on the Regulation of Apoptosis in Mouse Hepatocytes.

Conolly, R. B. Quantitative Modeling of Liver Foci in Rats Given Diethylnitrosamine (DEN) Followed by 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD): Alternate Models, with and without an Effect of TCDD on the Initiation Rate, Work Equally Well.

Constan, A. A., Butterworth, B. E., Wolf, D. C., Sprankle, C. S., Wong, B. A., and Kedderis, G. L. Metabolism of Chloroform by Cytochrome P450 Is Required for Induction of Toxicity in the Liver and Kidney of Male B6C3F1 Mice.

Constan, A. A., Wolf, D. C., Sprankle, C. S., Wong, B. A., and Butterworth B. E. Conditions Necessary for Inhaled Chloroform to Induce Regenerative Cell Proliferation in the Female B6C3F1 Mouse Liver.

Fennell, T. R. and MacNeela, J. P. Disposition and Metabolism of *p*-Nonylphenol in Male

and Female Rats.

Gaido, K. W., Leonard, L. S., Ramamoorthy, K.,* Wang, F.,* Chen, I.-C.,* Norris, J. D.,* McDonnell, D. P.,* Bocchinfus, W. P.,* Korach, K. S.,* and Safe, S.* Estrogenic Activity of a Dieldrin-Toxaphene Mixture in the Mouse Uterus, MCF-7 Breast Cancer Cells, and Yeast-Based Estrogen Receptor Assays: No Apparent Synergism.

Gonzales, A. J., Christensen, J., Goldsworthy, T. L., and Fox, T. R. Attenuation of G_1 Checkpoint Response in B6C3F1 Mouse Hepatocytes by Phenobarbital.

Gould, J. C., Leonard, L., Maness, S. C., McDonnell, D,* Conner, K.,* Zackarewski, T.,* Safe, S.,* and Gaido, K. W. Bisphenol A Interacts with the Estrogen Receptor in a Distinct Manner from Estradiol.

Healy, L. N., Romach, E. H., Fox, T. R., and Goldsworthy, T. L. Nongenotoxic and Genotoxic Chemically Induced Lesions Differ from Spontaneous Lesions in Mouse Liver.

Keys, D. A. and Conolly, R. B. A Physiologically Based Pharmacokinetic Model of di(2-Ethylhexyl) Phthalate and mono(2-Ethylhexyl) Phthalate in Adult and Juvenile Male Rats.

Lapinskas, P. J., Fen, L. Q.,* and Corton, J. C. Phthalate Ester Plasticizers Differentially Activate the Peroxisome Proliferator-Activated Receptors.

Maness, S. C., Leonard, L. S., and Gaido, K. W. 2′,4′,6′-Trichloro-4-Biphenyl and 2′,3′,4′,5′-Tetrachloro-4-Biphenyl Interactions in a Steroid-Response Reporter Gene Assay Using HepG2 Cells.

Mugford, C. A. and Kedderis, G. L. Role of Bioactivation in the Formation of DNA Double-Strand Breaks in Isolated Rat Hepatocytes.

Mylchreest, E. and Foster, P. M. D. Altered Reproductive Development in Male Rats Following In Utero and Lactational Exposure to di(n-Butyl) Phthalate.

Pastino, G. M., Roberts, K., James, A., Asgharian, B., and Bond, J. A. Pharmacokinetics of Inhaled Ethanol in Male and Female Rats and Mice.

Poet, T. S., Valentine, J. L., and Borghoff, S. J. Pharmacokinetics of *t*-Butyl Alcohol in F-344 Rats.

Prescott-Mathews, J. S., Wolf, D. C., Wong, B. A., and Borghoff, S. J. *tert*-Butyl Alcohol-Induced Protein Droplet Nephropathy in Male F-344 Rats.

Schlosser, P. M. and Lovern, M. R. Tentative Identification of Benzene Oxide In Vitro and In Vivo.

Selvaraj, L., Fennell, T. R., and Sumner, S. C. J. Characterization of Phosphodiester Adducts Produced by the Reaction of Ethylene Oxide with Nucleotides.

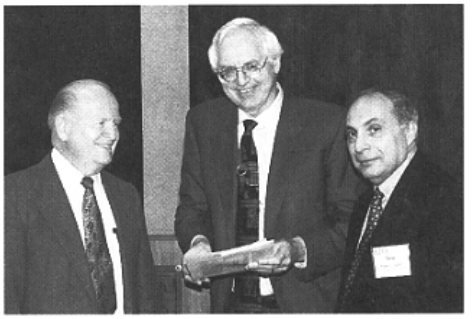
Sprankle, C. S., Templin, M. V., Constan, A. A., Wolf, D. C., Pluta, L., Recio, L., Wong, B., and Butterworth, B. E. Mutant Frequencies in Livers of Female *lacI* Transgenic Big Blue B6C3F1 Mice Following Chloroform Inhalation.

Sumner, S. C., Asgharian, B., and Fennell, T. R. Blood Pharmacokinetics of Tertiary Amyl Methyl Ether in Male and Female Rats and Mice Following Inhalation Exposure.

Vitarella, D., Struve, M. F., Goetz, J., Ledford, F. I., Miller, R., and Dorman, D. C. Comparative Neurotoxicity of Oral Manganese (II) Chloride in Neonatal and Adult CD Rats.

You, L., Sar, M., Archibeque-Engle, S., Casanova, M., and Heck, H. d'A. Transplacental and Lactational Transfer of *p,p′*-DDE and Effects on Androgen Receptor in the Epididymis and Testis of Sprague-Dawley Rats.

*Non-CIIT author.



Dr. Allen Lenz (left), CIIT President Dr. Roger McClellan, and CIIT Board Chairman Dr. Eugene Capaldi, ARCO Chemical Company.

Chemical Manufacturers Association Economist Speaks at CIIT

Dr. Allen J. Lenz, Director of Economic Analysis, Chemical Manufacturers Association, presented a public seminar at CIIT on February 11 in conjunction with the February 12 meeting of the CIIT Board of Directors. Dr. Lenz spoke on "The Chemical Industry: High-Tech Keystone of the U.S. Economy." In the seminar, he emphasized the economic contributions of the chemical industry and discussed the challenges to the industry that lie ahead in an increasingly competitive world economy.

Dr. Lenz holds a B.S. degree from the Wharton School of Finance and Commerce, University of Pennsylvania; an M.B.A. degree from the University of Colorado; and a Ph.D. degree from the Stanford Graduate School of Business. His professional experience includes 14 years of service with the Federal government in a variety of international trade and economic positions. He is the author of *Beyond Blue Economic Horizons: U.S. Trade Performance and International Competitiveness in the 1990s* (Praeger, 1991) as well as numerous other publications.

Michele Medinsky Appointed to EPA Environmental Health Committee

CIIT Senior Scientist Dr. Michele A. Medinsky was recently appointed as a full member of the Environmental Health Committee, Scientific Advisory Board (SAB), U.S. Environmental Protection Agency (EPA). Her appointment to the Environmental Health Committee extends through the end of September 1998.

The SAB consists of nongovernmental scientists and engineers who provide independent technical advice to EPA Administrator Carol Browner. SAB members advise the Agency on public health and environmental issues. The SAB, which plays an important role in the Agency's peer review process, reviews both research activities and regulatory issues. This year, for example, the Environmental Health Committee will be providing advice on the EPA's proposed Cancer Risk Assessment Guidelines.

Dr. Medinsky is internationally recognized for her research on incorporation of biochemical mechanisms into physiologically based pharmacokinetic models. These models assist in the understanding of exposure-dose relationships and interspecies extrapolation, critical issues that undergird the establishment of many human health risk standards.

Dr. Medinsky also serves on the Committee on Toxicology, National Research Council, National Academy of Sciences and on the Alcohol-Toxicology Study Section of the National Institutes of Health. She is currently President of the Inhalation Specialty Section of the Society of Toxicology.

CIIT President Dr. Roger O. McClellan, a past SAB member, is currently a consultant to the SAB. He served on the Environmental Health Committee when it reviewed the proposed Cancer Risk Assessment Guidelines and on the Clean Air Scientific Advisory Committee panels for ozone and particulate matter.

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Rohm and Haas Company
Schuller International, Inc.
Shell Chemical Company
Texaco Inc.
Union Carbide Corporation
Unocal Corporation
Volkswagen of America, Inc.
Vulcan Materials Company

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New CIIT Board member Mr. Marc G. Schurger (left) and member company representative Dr. W. Mills Dyer, Jr. (center), both of Eastman Chemical Company, with Mr. David S. Althaus, CIIT Controller and Manager of Financial Services.

Dr. Joseph A. LoMenzo (left), who represented Novartis Corporation at the February 12th meeting of the CIIT Board of Directors, with Dr. Roger O. McClellan, President of CIIT.

CIIT Board of Directors Announces a New Member Company and New Board Members

The CIIT Board of Directors approved Owens Corning Corporation as the Institute's newest member company at its meeting on February 12. Owens Corning, a world leader in high-performance glass composites and building materials, will be represented on the CIIT Board by Joel R. Bender, Ph.D., M.D., Vice President of Health Sciences and Chief Medical Officer.

Mr. Marc G. Schurger, Director of Product Safety and Regulatory Programs at Eastman Chemical Company, is also new to the CIIT Board of Directors. A third new Board member will represent Novartis Corporation, a producer of agricultural chemicals and pharmaceuticals. He is Mr. Ray H. Ankers, Vice President of Health, Safety and Environment.