Endocrine Reviews. First published ahead of print March 14, 2012 as doi:10.1210/er.2011-1050



# Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses

Laura N. Vandenberg, Theo Colborn, Tyrone B. Hayes, Jerrold J. Heindel, David R. Jacobs, Jr., Duk-Hee Lee, Toshi Shioda, Ana M. Soto, Frederick S. vom Saal, Wade V. Welshons, R. Thomas Zoeller, and John Peterson Myers

Center for Regenerative and Developmental Biology and Department of Biology (L.N.V.), Tufts University, Medford, Massachusetts 02155; The Endocrine Disruption Exchange (T.C.), Paonia, Colorado 81428; Laboratory for Integrative Studies in Amphibian Biology (T.B.H.), Molecular Toxicology, Group in Endocrinology, Energy and Resources Group, Museum of Vertebrate Zoology, and Department of Integrative Biology, University of California, Berkeley, California 94720; Division of Extramural Research and Training (J.J.H.), National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, Research Triangle Park, North Carolina 27709; Division of Epidemiology and Community Health (D.R.J.), School of Public Health, University of Minnesota, Minneapolis, Minnesota 55455; Department of Preventive Medicine (D.-H.L.), School of Medicine, Kyungpook National University, Daegu 702-701, Korea; Molecular Profiling Laboratory (T.S.), Massachusetts General Hospital Center for Cancer Research, Charlestown, Massachusetts 02129; Department of Anatomy and Cellular Biology (A.M.S.), Tufts University School of Medicine, Boston, Massachusetts 02111; Division of Biological Sciences (F.S.v.S.) and Department of Biomedical Sciences (W.V.W.), University of Missouri-Columbia, Columbia, Missouri 65211; Biology Department (T.Z.), University of Massachusetts-Amherst, Amherst, Massachusetts 01003; and Environmental Health Sciences (J.P.M.), Charlottesville, Virginia 22902

For decades, studies of endocrine-disrupting chemicals (EDCs) have challenged traditional concepts in toxicology, in particular the dogma of "the dose makes the poison," because EDCs can have effects at low doses that are not predicted by effects at higher doses. Here, we review two major concepts in EDC studies: low dose and nonmonotonicity. Low-dose effects were defined by the National Toxicology Program as those that occur in the range of human exposures or effects observed at doses below those used for traditional toxicological studies. We review the mechanistic data for low-dose effects and use a weight-of-evidence approach to analyze five examples from the EDC literature. Additionally, we explore nonmonotonic dose-response curves, defined as a nonlinear relationship between dose and effect where the slope of the curve changes sign somewhere within the range of doses examined. We provide a detailed discussion of the mechanisms responsible for generating these phenomena, plus hundreds of examples from the cell culture, animal, and epidemiology literature. We illustrate that nonmonotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. Whether low doses of EDCs influence certain human disorders is no longer conjecture, because epidemiological studies show that environmental exposures to EDCs are associated with human diseases and disabilities. We conclude that when nonmonotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. Thus, fundamental changes in chemical testing and safety determination are needed to protect human health. (***Endocrine Reviews*** **33: 0000–0000, 2012)**

I. Introduction
  A. Background: low-dose exposure
  B. Background: NMDRCs
  C. Low-dose studies: a decade after the NTP panel's assessment
  D. Why examine low-dose studies now?
  E. Mechanisms for low-dose effects
  F. Intrauterine position and human twins: examples of natural low-dose effects
II. Demonstrating Low-Dose Effects Using a WoE Approach
  A. Use of a WoE approach in low-dose EDC studies
  B. Refuting low-dose studies: criteria required for acceptance of studies that find no effect
  C. BPA and the prostate: contested effects at low doses?
  D. BPA and the mammary gland: undisputed evidence for low-dose effects

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
Copyright © 2012 by The Endocrine Society
doi: 10.1210/er.2011-1050 Received October 27, 2011. Accepted February 7, 2012.

Abbreviations: A4, Androstenedione; AhR, aryl hydrocarbon receptor; BPA, bisphenol A; CDC, Centers for Disease Control and Prevention; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane;DES, diethylstilbestrol; EDC, endocrine-disrupting chemicals; EPA, Environmental Protection Agency; ER, estrogen receptor; FDA, Food and Drug Administration; GLP, good laboratory practices; LOAEL, lowest observed adverse effect level; mER, membrane-associated ER; NHANES, National Health and Nutrition Examination Survey; NIS, sodium/iodide symporter; NMDRC, nonmonotonic dose-response curve; NOEL, no observed effect level; NOAEL, no observed adverse effect level; NTP, National Toxicology Program; PIN, prostatic intraepithelial neoplasias; POP, persistent organic pollutants; ppb, parts per billion; SERM, selective ER modulator; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; WoE, weight of evidence.



Copyright (C) 2012 by The Endocrine Society

E. Another controversial low-dose example: atrazine and amphibian sexual development
F. Dioxin and spermatogenesis: low-dose effects from the most potent endocrine disruptor?
G. Perchlorate and thyroid: low-dose effects in humans?
H. Low-dose summary
III. Nonmonotonicity in EDC Studies
A. Why is nonmonotonicity important?
B. Mechanisms for NMDRCs
C. Examples of nonmonotonicity
D. NMDRC summary
IV. Implications of Low-Dose Effects and Nonmonotonicity
A. Experimental design
B. Regulatory science
C. Human health
D. Wildlife
V. Summary

## I. Introduction

This review focuses on two major issues in the study of endocrine-disrupting chemicals (EDCs): low-dose exposures and nonmonotonic dose-response curves (NMDRCs). These concepts are interrelated, and NMDRCs are especially problematic for assessing potential impacts of exposure when nonmonotonicity is evident at levels of exposure below those that are typically used in toxicological assessments. For clarity of presentation, however, we will first examine each of the concepts separately.

### A. Background: low-dose exposure

It is well established in the endocrine literature that natural hormones act at extremely low serum concentrations, typically in the picomolar to nanomolar range. Many studies published in the peer-reviewed literature document that EDCs can act in the nanomolar to micromolar range, and some show activity at picomolar levels.

#### *1. What is meant by low dose?*

In 2001, at the request of the U.S. Environmental Protection Agency (EPA), the National Toxicology Program (NTP) assembled a group of scientists to perform a review of the low-dose EDC literature (1). At that time, the NTP panel defined low-dose effects as any biological changes 1) occurring in the range of typical human exposures or 2) occurring at doses lower than those typically used in standard testing protocols, *i.e.* doses below those tested in traditional toxicology assessments (2). Other definitions of low dose include 3) a dose below the lowest dose at which a biological change (or damage) for a specific chemical has been measured in the past, *i.e.* any dose below the lowest observed effect level or lowest observed adverse effect level (LOAEL) (3), or 4) a dose administered to an animal that produces blood concentrations of that chemical in the range of what has been measured in the general human population (*i.e.* not exposed occupationally, and often referred to as an environmentally relevant dose because it creates an internal dose relevant to concentrations of the chemical measured in humans) (4, 5). This last definition takes into account differences in chemical metabolism and pharmacokinetics (*i.e.* absorption, distribution, and excretion of the chemical) across species and reduces the importance of route of exposure by directly comparing similar blood or other tissue concentrations across model systems and experimental paradigms. Although these different definitions may seem quite similar, using just a single well-studied chemical like bisphenol A (BPA) shows how these definitions produce different cutoffs for exposure concentrations that are considered low dose (Table 1). For many chemicals, including EDCs, a large number of studies meet the criteria for low-dose studies regardless of whether the cutoff point for a low dose was based on the range of typical human exposures, doses used in traditional toxicology, or doses that use an internal measure of body burden.

Whether low doses of EDCs influence disease is a question that now extends beyond the laboratory bench, because epidemiological studies show that environmental exposures to these chemicals are associated with disorders in humans as well (see for examples Refs. 6–16). Although disease associations have historically been observed in individuals exposed to large concentrations of EDCs after

**TABLE 1.** Low-dose definitions and cutoff doses: BPA and DEHP as examples

| Chemical | Estimated range of human exposures | Doses below the NOAEL | Doses below the LOAEL | Administered doses (to animals) that produce blood levels in typical humans |
|---|---|---|---|---|
| BPA | 0.4–5 $\mu$g/kg · d (679) | No NOAEL was ever established in toxicological studies (38) | <50 mg/kg · d (38) | ~400 $\mu$g/kg · d to rodents and nonhuman primates (4, 253) |
| DEHP | 0.5–25 $\mu$g/kg · d (680) | <5.8 mg/kg · d (681, 682) | <29 mg/kg · d (681, 682) | Unknown |

Estimates of human exposure are made from consumer product consumption data but do not take into account that there are unknown sources of these chemicals.
DEHP, Bis(2-ethylhexyl) phthalate.

industrial accidents (17–19) or via occupational applications (20–22), recent epidemiological studies reveal links between environmentally relevant low concentrations and disease prevalence. With the extensive biomonitoring studies performed by the U.S. Centers for Disease Control and Prevention (CDC) (23, 24) and similar environmental surveys performed in Europe (25) and elsewhere (www.statcan.gc.ca/concepts/hs-es/measures-mesures-eng.htm), knowledge about environmental exposures to EDCs and their associations with human health disorders has increased substantially.

Low-dose effects have received considerable attention from the scientific and regulatory communities, especially when examined for single well-studied chemicals like BPA (4, 27–32). The low-dose literature as a whole, however, has not been carefully examined for more than a decade. Furthermore, this body of literature has been disregarded or considered insignificant by many (33, 34). Since the NTP's review of the low-dose literature in 2001 (2), a very large body of data has been published including 1) additional striking examples of low-dose effects from exposures to well-characterized EDCs as well as other chemicals, 2) an understanding of the mechanisms responsible for these low-dose effects, 3) exploration of nonmonotonicity in *in vivo* and *in vitro* systems, and 4) epidemiological support for both low-dose effects and NMDRCs.

#### *2. Is the term low dose a misnomer?*

Endogenous hormones are active at extremely low doses, within and below the picomolar range for endogenous estrogens and estrogenic drugs, whereas environmental estrogen mimics are typically active in the nanomolar to micromolar range (for examples, see Refs. 35–38), although some show effects at even lower concentrations (39–41). Importantly, the definitions above do not take into account the potency or efficacy of the chemical in question, a topic that will be discussed in greater detail below. Instead, low dose provides an operational definition, in which doses that are in the range of human exposure, or doses below those traditionally tested in toxicological studies, are considered low. To be clear, none of these definitions suggest that a single concentration can be set as a low dose cutoff for all chemicals. Using the above definitions, for some chemicals, low doses could potentially be in the nanogram per kilogram range, but for most chemicals, doses in the traditional micro- and milligram per kilogram range could be considered low doses because traditional approaches to testing chemicals typically did not examine doses below the milligram per kilogram dose range.

### B. Background: NMDRCs

We have defined low-dose studies according to the definitions established by the NTP panel of experts (2). However, because the types of endpoints that are typically examined at high doses in toxicological studies are often different from the types of endpoints examined in low-dose studies, one cannot assume that an effect reported in the low-dose range is necessarily different from what would be observed at higher doses. For example, low doses of a chemical could affect expression of a hormone receptor in the hypothalamus, an endpoint not examined in high-dose toxicology testing, and high doses could similarly affect this same endpoint (but are likely to be unreported because high doses are rarely tested for these types of endpoints). Thus, the presence of low-dose effects makes no assumptions about what has been observed at higher concentrations. (As discussed elsewhere, for the majority of chemicals in commerce, there are no data on health effects and thus no established high- or low-dose range.) Therefore, low-dose effects could be observed at the lower end of a monotonic or linear dose-response curve.

In contrast, the definition of a NMDRC is based upon the mathematical definition of nonmonotonicity: that the slope of the dose-response curve changes sign from positive to negative or vice versa at some point along the range of doses examined (42). Often NMDRCs have a U- or inverted U-shape (43); these NMDRCs are thus also often referred to as biphasic dose-response curves because responses show ascending and descending phases in relation to dose. Complex, multiphasic curves have also been observed (41, 44, 45). NMDRCs need not span from true low doses to high (pharmacologically relevant) doses, although experiments with such a broad dose range have been performed for several EDCs; the observation of nonmonotonicity makes no assumptions about the range of doses tested. Examples of NMDRCs from *in vitro* cell culture and *in vivo* animal experiments, as well as epidemiological examples, are presented in detail later in this review (see *Sections III.C.1–3*). Additional examples of NMDRCs are available in studies examining the effects of vitamins and other essential elements on various endpoints (see for example (46); these will not be examined in detail in this review due to space constraints.

NMDRCs present an important challenge to traditional approaches in regulatory toxicology, which assume that the dose-response curve is monotonic. For all monotonic responses, the observed effects may be linear or nonlinear, but the slope does not change sign. This assumption justifies using high-dose testing as the standard for assessing chemical safety. When it is violated, high-dose testing regimes cannot be used to assess the safety of low doses.

It should be noted that both low dose and nonmonotonicity are distinguished from the concept of hormesis, which is defined as a specific type of response whereby "the various points along [the dose response] curve can be interpreted as beneficial or detrimental, depending on the biological or ecological context in which they occur" (47). Estimations of beneficial or adverse effects cannot be ascertained from the direction of the slope of a dose-response curve (48–50). In their 2001 Low Dose Peer Review, the NTP expert panel declined to consider whether any effect was adverse because "in many cases, the long-term health consequences of altered endocrine function during development have not been fully characterized" (2). There are still debates over how to define adverse effects (51–53), so for the purposes of this review, we consider any biological change to be an effect. Importantly, most epidemiological studies are by definition examining low doses (unless they are focusing on occupationally exposed individuals), and these studies typically focus on endpoints that are accepted to be adverse for human health, although some important exceptions exist (54–56).

Finally, it is worth noting that any biological effect, whether it is observed to follow linear relationships with administered dose or not, provides conclusive evidence that an EDC has biological activity. Thus, other biological effects are likely to be present but may remain undetected or unexamined. Many EDCs, including those used as pesticides, were designed to have biological effects (for example, insecticides designed to mimic molting hormone). Thus, the question of whether these chemicals have biological effects is answered unequivocally in their design; the question is what other effects are induced by these biologically active agents, not whether they exist.

### C. Low-dose studies: a decade after the NTP panel's assessment

In 2000, the EPA requested that the NTP assemble a panel of experts to evaluate the scientific evidence for low-dose effects and dose-response relationships in the field of endocrine disruption. The EPA proposed that an independent and open peer review of the available evidence would allow for a sound foundation on which the EPA could "determine what aspects, if any, of its standard guidelines for reproductive and developmental toxicity testing [would] need to be modified to detect and characterize low-dose effects" (2). The NTP panel verified that low-dose effects were observed for a multitude of endpoints for specific EDCs including diethylstilbestrol (DES), genistein, methoxychlor, and nonylphenol. The panel identified uncertainties around low-dose effects after exposure to BPA; although BPA had low-dose effects on some endpoints in some laboratories, others were not found to be consistent, leading the panel to conclude that it was "not persuaded that a low-dose effect of BPA has been conclusively established as a general or reproducible finding" (2).

Since the NTP's review of low-dose endocrine disruptor studies, only a few published analyses have reexamined the low-dose hypothesis from a broad perspective. In 2002, R. J. Witorsch (57) analyzed low doses of xenoestrogens and their relevance to human health, considering the different physiologies associated with pregnancy in the mouse and human. He proposed that low doses of endocrine disruptors would not likely affect humans because, although low-dose effects had been observed in rodents, the hormonal milieu, organs controlling hormonal release, and blood levels of estrogen achieved are quite different in humans. There are, of course, differences in hormones and hormone targets between rodents and humans (58), but the view that these differences negate all knowledge gained from animal studies is not supported by evolutionary theory (59–61). This human-centered stance argues against the use of animals for any regulatory testing (62) and runs counter to the similarities in effects of EDCs on humans and animals; rodents proved to be highly predictive of the effects of DES on humans (63, 64). In a striking example, studies from mice and rats predicted that gestational exposure to DES would increase mammary cancer incidence decades before women exposed *in utero* reached the age where this increase in risk was actually observed (65–67).

In 2007, M. A. Kamrin (68) examined the low-dose literature, focusing on BPA as a test case. He suggested that three criteria were required to support the low-dose hypothesis. First is reproducibility, which he defined as "the same results are seen from the same causes each time a study is conducted." Furthermore, he proposed that the dose response for the effects must be the same from study to study. Second is consistency, which he defined as the results all fitting into a pattern, whereby the results collected from multiple species and under variable conditions all show the same effect. And third is proper conduct of studies, which he defined as including the appropriate controls and performance under suitable experimental conditions as well as the inclusion of multiple doses such that a dose-response curve can be obtained.

Although we and others (69–72) agree with the use of these criteria (reproducibility, consistency, and proper experimental design), there are significant weaknesses in the logic Kamrin employed to define these factors. First, suggesting that reproducibility is equivalent to the same results obtained each time a study is conducted is unrealistic and not a true representation of what is required of replication. As has been discussed in other fields, "there is no

end to the ways in which any two experiments can be counted as the same — or different . . . All experiments are the same in respect of their being experiments; they are all different by virtue of being done at different places, at different times, by different people, with different strains of rat, training regime, and so on" (73).

Furthermore, according to the Bradford-Hill criteria, a set of requirements accepted in the field of epidemiology to provide adequate evidence of a causal relationship between two factors, a single negative result (or even several studies showing negative results) cannot negate other studies that show adverse effects (74). Essentially, all scientists know that it is very easy for an experiment to find no significant effects due to a myriad of reasons; it is more difficult to actually find effects, particularly when using highly sophisticated techniques (69).

Second, the concept of consistency as a pattern that can be derived from all results is one we will use below, using a weight-of-evidence (WoE) approach and several specific examples. However, Kamrin's proposed idea that every study must show the same effect has the same weaknesses as discussed for the proposed definition of reproducibility and does not acknowledge the obvious differences in many species and strains. It also suggests that the identification of a single insensitive strain could negate any number of positive studies conducted with appropriate animal models (75).

And finally, Kamrin suggested that only studies with appropriate controls should be used for analyses, a criterion we agree should be followed. However, his own scrutiny of the low-dose animal literature fails to do so (68). He also suggested that studies use multiple doses so that a dose-response curve can be obtained. Although studies using a single dose can be informative, we agree that dose-response relationships provide important information to researchers and risk assessors alike. However, this requirement is not helpful if there is an insistence on observing a linear response; as we discuss in depth in this review, there are hundreds of examples of nonmonotonic and other nonlinear relationships between dose and endpoint. These should not be ignored.

In 2004, Hayes (76) reviewed the available literature concerning the effects of atrazine on amphibian development, with a specific focus on the effect of ecologically relevant doses of this EDC on malformations of the gonads and other sexually dimorphic structures; in the case of aquatic exposures, it can be difficult to determine what a cutoff for a low dose would be; thus, Hayes focused on studies examining the effects of atrazine at levels that had been measured in the environment. He reviewed the results produced by several labs, in which it was independently demonstrated that low concentrations of atrazine produced gonadal abnormalities including hermaphroditism, males with extra testes, discontinuous gonads, and other defects. Hayes' work also clearly addressed the so-called irreproducibility of these findings by analyzing the studies that were unable to find effects of the pesticide; he noted that the negative studies had multiple experimental flaws, including contamination of the controls with atrazine, overcrowding (and therefore underdosing) of experimental animals, and other problems with animal husbandry that led to mortality rates above 80%.

In 2006, vom Saal and Welshons (77) examined the low-dose BPA literature, identifying more than 100 studies published as of July 2005 that reported significant effects of BPA below the established LOAEL, of which 40 studies reported adverse effects below the 50 $\mu$g/kg · d safe dose set by the EPA and U.S. Food and Drug Administration (FDA); all of these studies would be considered low dose according to the NTP's definition (2). The authors proposed that these examples should be used as evidence to support the low-dose hypothesis. Furthermore, this publication detailed the similarities among the studies that were unable to detect any effects of low doses of BPA and established a set of criteria required to accept negative studies. We have adapted the criteria detailed by Hayes (76) and vom Saal and Welshons (77) to produce a set of requirements for low-dose studies; these criteria are described in some detail below.

### D. Why examine low-dose studies now?

The developmental origins of health and disease hypothesis originated from studies showing that fetal DES exposure could cause severe malformations and cancers of the reproductive tract, and other studies demonstrating that fetal malnutrition could lead to adult diseases including metabolic syndrome, diabetes, and increased stroke incidence (78–81). Since that time, the developmental origins of health and disease hypothesis has been extended to address whether diseases that are increasing in prevalence in human populations could be caused by developmental exposures to EDCs (67, 82–85). Evidence from the animal literature has been tremendously informative about the effects of EDC exposures early in development and has driven new hypotheses to be tested in epidemiology studies (86). Studies including several discussed in this review provide supportive evidence that the fetal and neonatal periods are specifically sensitive to chemicals that alter endocrine signaling and that EDCs could be contributing to a range of diseases.

Strong, reliable, and reproducible evidence documents the presence of low concentrations of EDCs and other chemicals in human tissues and fluids, as well as in environmental samples (28, 87–89). These studies indicate

that samples collected from humans and the environment typically contain hundreds of contaminants, usually in the parts-per-billion (ppb) range (90, 91). The obvious question with potentially large public health implications is whether these concentrations are so low as to be irrelevant to human health. The fact that epidemiological analyses (reviewed in *Section III.C.3*) repeatedly find associations between the measured concentrations in human samples and disease endpoints suggests it is inappropriate to assume the exposures are too low to matter. That is especially the case given the empirical data (reviewed in *Section II.A*) from animal and cell culture experiments showing effects can be caused by concentrations comparable (and sometimes below) what is measured in humans and also the detection of NMDRCs in some of those same experiments.

In the human biomonitoring field, large databases such as the CDC's National Health and Nutrition Examination Survey (NHANES) have allowed researchers to make comparisons between groups of individuals with various exposure criteria; some of these studies will be addressed in detail in subsequent sections of this review. Although by definition these databases examine low-dose exposures, their use has been the subject of significant debate. Because of the large number of chemicals that have been measured (>300 in the most recent NHANES by the CDC) and the large number of health outcomes and other disease-related data collected from the individuals that donated biological samples, it has been argued that the number of possible associations that could be made would lead to a significant number of false positives (92); thus, associations could be found simply because of extensive data dredging. This has led some to suggest that these studies as a whole should be rejected (93, 94).

In response to these criticisms, epidemiologist Jan Vandenbroucke (95) notes, "researchers do not mindlessly grind out one analysis after another"; the examination of these databases for associations between chemical exposures and health effects does not entail the statistical comparison between all possible factors, calculated as some 8800 comparisons in the CDC's NHANES database (92). Instead, epidemiologists typically focus on a select number of comparisons that address relationships between chemicals and diseases identified *a priori* (96, 97), often because of mechanistic data obtained in laboratory animals or *in vitro* work with human and animal cells and tissues. Repeated findings of links between EDC exposures and diseases in epidemiological analyses of biomonitoring data based on *a priori* hypotheses suggests these relationships should not be rejected as a statistical artifact and, instead, should be the basis for significant concern that low-dose effects can be detected in the general population (85, 98).

### E. Mechanisms for low-dose effects

The endocrine system is particularly tuned to respond to very low concentrations of hormone, which allows an enormous number of hormonally active molecules to coexist in circulation (38). As a ligand-receptor system, hormones act by binding to receptors in the cell membrane, cytosol, or the nucleus. The classical effects of nuclear hormone receptors influence gene expression directly, although rapid nongenomic actions at membrane-associated receptors are now well documented and accepted. Membrane receptors are linked to different proteins in the cell, and binding to these receptors typically changes cellular responses in a rapid fashion (99), although the consequence of a rapid signaling event could be the activation of a nuclear transcription factor, leading to responses that take longer to detect. Peptide hormones can also influence gene expression directly (see Refs. 100 and 101 for examples).

There are several means by which the endocrine system displays specificity of responses to natural hormones. Many hormone receptors are expressed specifically in a single or a few cell types (for example, receptors for TSH are localized to the thyroid), whereas some (like thyroid hormone receptors) are found throughout the body (102). For receptors that are found in multiple cell types, different effects are produced in part due to the presence of different coregulators that influence behaviors of the target genes (103–105). And finally, some hormones have multiple receptors [for example estrogen receptor (ER)$\alpha$ and ER$\beta$], which are expressed in different quantities in different cell types and organs and can produce variable effects on gene expression or cellular phenomena (cell proliferation *vs.* apoptosis) (102, 106).

The typical physiological levels of the endogenous hormones are extremely low, in the range of 10–900 pg/ml for estradiol, 300–10,000 pg/ml for testosterone, and 8–27 pg/ml for $T_4$ (see Table 2). Importantly, steroid hormones in the blood are distributed into three phases: free, representing the unconjugated, unbound form; bioavailable, representing hormones bound to low-affinity carrier proteins such as albumin; and inactive, representing the form that is bound to high-affinity binding proteins such as SHBG or $\alpha$-fetoprotein (38) (Fig. 1A). When the circulating levels in blood are corrected for the low fraction of the hormones that are not bound to serum binding proteins, the free concentrations that actually bring about effects in cells are even lower, for example 0.1–9 pg/ml for estradiol. Concentrations of active hormones will vary based on the age and physiological status of the individual (*i.e.* plasma testosterone levels are less than 1 ng/ml in male children but increase to approximately 5–7 ng/ml in adulthood; during menses, estradiol levels are typically less than 100

**TABLE 2.** Ranges of endogenous hormones in humans (from Ref. 108)

| Hormone | Free concentration (females) | Total concentration (females) | Free concentration (males) | Total concentration (males) |
|---|---|---|---|---|
| Cortisol | 20–300 ng/ml | | 20–300 ng/ml | |
| Estradiol | 0.5–9 pg/ml (adult female) | <20 pg/ml (prepubertal)<br>20–800 pg/ml (premenopausal)<br><30 pg/ml (postmenopausal) | | 10–60 pg/ml (adult) |
| Progesterone | | 0.2–0.55 ng/ml (prepubertal)<br>0.02–0.80 ng/ml (follicular phase)<br>0.90–4 ng/ml (luteal phase)<br><0.5 ng/ml (postmenopausal) | | 0.1–0.4 ng/ml (prepubertal)<br>0.2–2 ng/ml (adult) |
| Insulin | | 0–250 pmol/liter | | 0–250 pmol/liter |
| GH | | 2–6 ng/ml | | 2–6 ng/ml |
| Prolactin | | 0–15 ng/ml | | 0–10 ng/ml |
| Testosterone | 9–150 pg/ml (adult) | | 0.3–250 ng/ml | |
| Thyroid hormone | 8–30 pg/ml (10–35 pM) | | 8–30 pg/ml (10–35 pM) | |
| TSH | 0.5–5 μU/ml | | 0.5–5 μU/ml | |

pg/ml, but just before ovulation, they spike to 800 pg/ml; *etc.*) (107, 108). Of course, it should be noted that active concentrations of natural hormones vary somewhat from species to species and can even vary between strains of the same species (109).

There are several reasons why endogenous hormones are able to act at such low circulating concentrations: 1) the receptors specific for the hormone have such high affinity that they can bind sufficient molecules of the hormone to trigger a response, 2) there is a nonlinear relationship between hormone concentration and the number of bound receptors, and 3) there is also a nonlinear relationship between the number of bound receptors and the strongest observable biological effect. Welshons and colleagues (38) describe how hormone concentration influences receptor occupancy: "receptor occupancy is never determined to be linear in relation to hormone concentration . . . At concentrations above the $K_d$ [the dissociation constant for receptor-ligand binding kinetics], saturation of the response occurs first, and then at higher concentrations, saturation of receptors is observed." What this means is that at low doses of hormone, a 10-fold increase in hormone concentration can have a 9-fold increase in receptor occupancy, whereas at high doses of hormone, a 10-fold increase in hormone concentration produces a less than 1.1-fold increase in receptor occupancy (38) (Fig. 1B). Thus, even moderate changes in hormone concentration in the low-dose range can produce substantial changes in receptor occupancy and therefore generate significant changes in biological effects. Welshons *et al.* (38) also note that a near-maximum biological response can be observed without a high rate of receptor occupancy, a situation that was previously termed the spare receptor hypothesis (110, 111); that is, the response mechanism saturates before all of the receptors are saturated. The presence of spare receptors is the basis for saying that these receptor systems are tuned to detect low concentrations that lead to occupancy of 0.1–10% of total receptors. Within this range of low receptor occupancy, there is high proportionality between changes in the free hormone concentration and changes in receptor occupancy, and a change in receptor occupancy by a ligand for the receptor is required to initiate changes in receptor-mediated responses (38).

There are additional reasons why natural hormones are active at low doses: 4) hormones have a strong affinity for their receptors (relative to affinity for other receptors) because many hormones are secreted from a single gland or site in the body but must have effects throughout the body in multiple tissues and 5) blood concentrations of hormones are normally pulsatile in nature, with the release of one hormone often controlled by the pulsatile release of another hormone (112, 113), and both the frequency and the amplitude of pulses modulate the biological response; hormones are also influenced by circadian rhythms, with dramatic differences in hormone secretion depending on the time of day (114, 115).

For many years, the mechanisms by which some environmental chemicals acted at low doses were not well understood. In 1995, the National Research Council appointed the Committee on Hormonally Active Agents in the Environment to address public concerns about the potential for adverse effects of EDCs on human health (116). At the time, work on understanding the mechanisms by which EDCs exert their effects was in its infancy, and in the executive summary, the committee stated, "Lack of knowledge about a mechanism does not mean that a reported effect is unconfirmed or unimportant, nor does demonstration of a mechanism document that the resulting effects are unique to that mechanism or are pervasive

**Figure 1.**

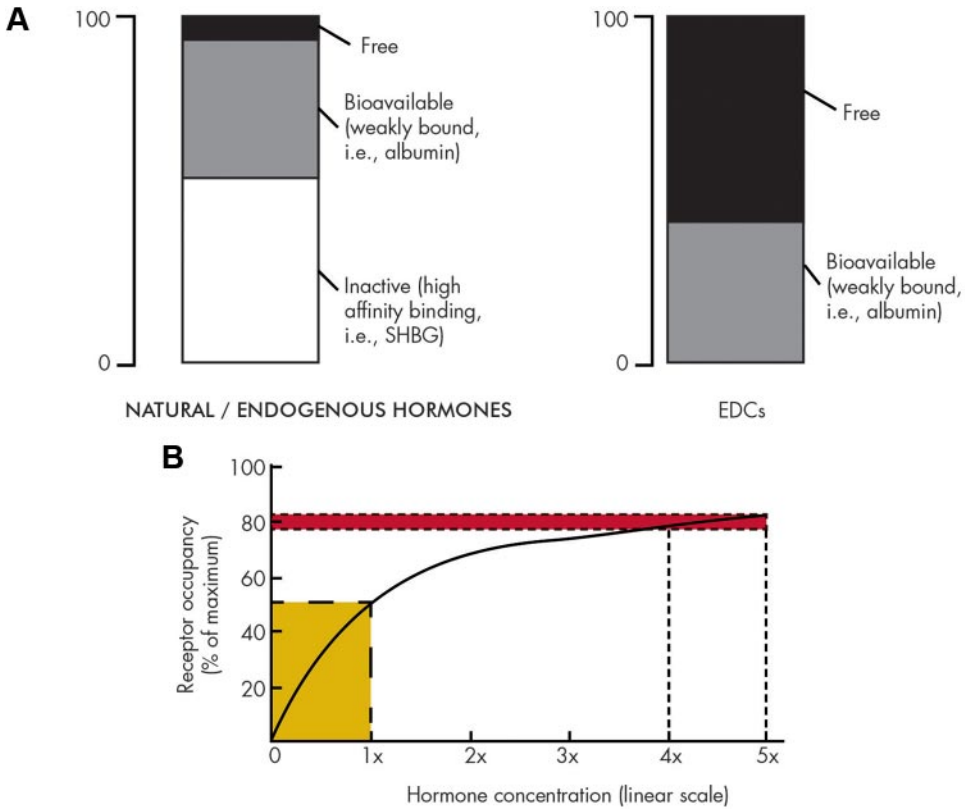



Figure 1. Characteristics and activities of natural hormones. A, This schematic depicts a typical relationship of three phases of circulating hormones: free (the active form of the hormone), bioavailable (bound weakly to proteins such as albumin), and inactive (bound with high affinity to proteins such as SHBG). These three phases act as a buffering system, allowing hormone to be accessible in the blood, but preventing large doses of physiologically active hormone from circulating. With EDCs, there may be little or no portion maintained in the inactive phase. Thus, the entirety or majority of a circulating EDC can be physiologically active; the natural buffering system is not present, and even a low concentration of an EDC can disrupt the natural balance of endogenous hormones in circulation. B, Schematic example of the relationship between receptor occupancy and hormone concentration. In this theoretical example, at low concentrations, an increase in hormone concentration of x (from 0 to 1x) causes an increase in receptor occupancy of approximately 50% (from 0 to 50%, see *yellow box*.) Yet the same increase in hormone concentration at higher doses (from 4x to 5x) causes an increase in receptor occupancy of only approximately 4% (from 78 to 82%, see *red box*).

in natural systems." Since that time, a tremendous amount of work has been dedicated to understanding the molecular mechanisms of action of EDCs, and in particular the mechanisms responsible for low-dose effects.

### *1. General mechanisms for EDC action*

As discussed above, the endocrine system evolved to function when unbound physiologically active ligands (hormones) are present at extremely low doses (117). Because of shared receptor-mediated mechanisms, EDCs that mimic natural hormones have been proposed to follow the same rules and therefore have biological effects at low doses (38, 118). Similarly, EDCs that influence in any way the production, metabolism, uptake, or release of hormones also have effects at low doses, because even small changes in hormone concentration can have biologically important consequences (38, 119).

The estrogen-response mechanisms have been extensively studied with regard to the effects of endogenous estrogens and estrogenic drugs. In classical, genomic estrogen action, when endogenous estrogens bind to ER, those receptors bind to estrogen response element sequences or to a number of other response element sites adjacent to the genes directly responsive to estrogens; this binding influences transcription of estrogen-sensitive genes (120). Xenoestrogens produce the same reactions; these chemicals bind to ERs, which then initiate a cascade of molecular effects that ultimately modify gene expression. Therefore, for the actions of estrogenic EDCs, molecular mechanisms and targets are already known in some detail. Similar mechanisms are induced by the binding of androgens to the androgen receptor, or thyroid hormone agonists to the thyroid hormone receptor, among others.

Additionally, there are EDCs that act as antagonists of these hormone systems, binding to a receptor, but not activating the receptor's typical response, and preventing the binding or activity of the endogenous ligand. Finally, many EDCs bind to the receptor and trigger a response that is not necessarily the same as that triggered by the endogenous estrogens; these are termed selective ER modulators (SERMs). Ultimately, all of these actions occur at the level of the receptor.

Many studies have been dedicated to the understanding of which EDCs bind to which nuclear hormone receptors and how the binding affinities compare to the natural steroid. Thus, many of these chemicals have been classified as weak hormones. Yet studies have shown that, for example, the so-called weak estrogens like BPA can be equally potent as endogenous hormones in some systems, causing biological effects at picomolar levels (30, 38, 41, 121). Both endogenous estrogens and EDCs can bind to ER associated with the cell membrane [membrane-associated ER (mER)$\alpha$ and mER$\beta$] that are identical to the nuclear ER (122–124), and a transmembrane ER called G-protein coupled receptor 30 that is structurally dissimilar to the nuclear ER and encoded by a distinct gene (125, 126). In many cells, 5–10% of total ER$\alpha$ and ER$\beta$ are localized to the plasma membrane (124); these membrane-associated receptors are capable of nongenomic steroid action in various cell types (30, 121, 127); thus, rapid and potent effects are well documented for many EDCs including BPA, DES, endosulfan, dichlorodiphenyldichloroethylene (DDE), dieldrin, and nonylphenol, among others (41, 128–130).

Finally, EDCs have other effects that are not dependent on binding to either classical or membrane-bound steroid hormone receptors. EDCs can influence the metabolism of natural hormones, thus producing differences in the amount of hormone that is available for binding either because more (or less) hormone is produced than in a typical system or because the hormone is degraded faster (or slower) than is normal. Other EDCs influence transport of hormone, which can also change the amount of hormone that is available for receptor binding. And EDCs can also have effects that are independent from known endocrine actions. One example is the effect of endogenous hormones and EDCs on ion channel activity. BPA, dichlorodiphenyltrichloroethane (DDT), DES, nonylphenol, and octylphenol have all been shown to disrupt $Ca^{2+}$ channel activity and/or $Ca^{2+}$ signaling in some cell types (131–134). This example illustrates how both natural hormones and EDCs can have hormonal activity via binding to nuclear hormone receptors but may also have unexpected effects via receptor-mediated actions outside of the classical endocrine system.

### *2. Mechanisms of EDC-induced low-dose actions*

The various mechanisms by which EDCs act *in vitro* and *in vivo* provide evidence to explain how these chemicals induce effects that range from altered cellular function, to abnormal organ development, to atypical behaviors. Just as natural hormones display nonlinear relationships between hormone concentration and the number of bound receptors, as well as between the number of bound receptors and the maximal observable biological effect, EDCs obey these rules of binding kinetics (38). Thus, in a way, EDCs exploit the highly sensitive endocrine system and produce significant effects at relatively low doses.

To gain insight into the effects of natural hormones and EDCs on gene expression profiles, it is possible to calculate doses that produce the same effect on proliferation of cultured cells, *i.e.* the quantitative cellular response doses, and determine the effect of those doses on transcriptomal signature profiles. When this is done for estradiol and EDCs with estrogenic properties, the affected estrogen-sensitive genes are clearly different (135). However, an interesting pattern emerges: comparing profiles among only the phytoestrogens shows striking similarities in the genes up- and down-regulated by these compounds; profile comparisons between only the plastic-based estrogens also show similarities within this group. Yet even more remarkable is what occurs when the doses are selected not based on cell proliferation assays but instead on the ability of estradiol and estrogen-mimics to induce a single estrogen-sensitive marker gene. When doses were standardized based on marker gene expression, the transcriptomal signature profiles were very similar between estradiol and estrogen mimics (135). Taken together, these results suggest that the outcomes of these experiments are contextual to the normalization parameter and that marker gene expression and cell proliferation are not superimposable. This indicates that the biological level at which the effects of chemicals are examined (*i.e.* gene expression, cellular, tissue, organ, or organismal) can greatly impact whether low-dose effects are observed and how these effects are interpreted.

There are several other mechanisms by which low-dose activities have been proposed. One such possibility is that low doses of EDCs can influence the response of individuals or organs/systems within the body to natural hormones; thus, the exposed individual has an increased sensitivity to small changes in endogenous steroids, similar to the effects of intrauterine position (see Ref. 136 and *Section I.F*). In fact, several studies have shown that exposure to EDCs such as BPA during perinatal development can influence the response of the mammary gland to estrogen (137, 138) and the prostate to an estrogen-testosterone

mixture similar to the concentrations produced in aging men (139–142). There is also evidence that EDCs work additively or even synergistically with other chemicals and natural hormones in the body (143–145). Thus, it is plausible that some of the low-dose effects of an EDC are actually effects of that exogenous chemical plus the effects of endogenous hormone.

Finally, it should be noted that during early development, the rodent fetus is largely, but not completely (146), protected from estrogen via the binding activity of $\alpha$-fetoprotein, a plasma protein produced in high levels by the fetal liver (147). Some estrogen-like EDCs, however, bind very weakly to $\alpha$-fetoprotein, and therefore, it is likely that this protein does not provide protection to the fetus during these sensitive developmental periods (36, 148). Furthermore, because EDCs may not bind to $\alpha$-fetoprotein or other high-affinity proteins in the blood (148–150) and can have a higher binding affinity to proteins like albumin (compared with natural estrogens) (36, 149), the balanced buffer system in place for endogenous hormones may be disturbed (Fig. 1A). Thus, whereas only a portion of endogenous hormones are bioavailable, the entirety of a circulating EDC could be physiologically active.

The effects of hormones and EDCs are dependent on dose, and importantly, low (physiological) doses can be more effective at altering some endpoints compared with high (toxicological) doses. There are many well-characterized mechanisms for these dose-specific effects including signaling via single *vs.* multiple steroid receptors due to nonselectivity at higher doses (30), receptor down-regulation at high doses *vs.* up-regulation at low doses (151, 152), differences in the receptors present in various tissues (153, 154), cytotoxicity at high doses (155), and tissue-specific components of the endocrine-relevant transcriptional apparatus (104, 105). Some of these factors will be addressed in *Section III.B* in the section dedicated to NMDRCs.

### F. Intrauterine position and human twins: examples of natural low-dose effects

Hormones have drastically different effects at different periods of development. In a now classical *Endocrinology* paper, Phoenix and colleagues (156) showed that hormone exposures during early development, and in particular fetal development, had organizational effects on the individual, whereby the developing organs were permanently reorganized by exposure to steroids. Permanent, nonreversible masculinization of the developing body plan by androgen exposure *in utero* is an example. These organizational effects are in contrast to the effects of the same hormones, at similar or even higher doses, on adults. The effects of steroids on individuals after puberty have been termed activational, because the effects on target organs are typically transient; withdrawal of the hormone returns the phenotype of the individual to the preexposed state (157), although this is not always the case (158).

One of the most striking examples of the ability of low doses of hormones to influence a large repertoire of phenotypes is provided by the study of intrauterine positioning effects in rodents and other animals. The rodent uterus in particular, where each fetus is fixed in position along a bicornate uterus with respect to its neighbors, is an excellent model to study how hormones released from neighboring fetuses (159) can influence the development of endocrine-sensitive endpoints (31). Importantly, differences in hormonal exposures by intrauterine position are relatively small (see Fig. 2) (160). Thus, even a small magnitude in differences of hormonal exposures is sufficient to generate effects on behavior, physiology, and development.

The earliest studies of intrauterine position compared behavioral characteristics of females relative to their position in the uterus (161–164); male behavior was also affected by intrauterine position (161, 165–167). Subsequent studies of intrauterine position showed that position in the uterus influenced physiological endpoints (157, 160–162, 168–174) as well as morphological endpoints in female rodents (160, 161, 163, 164, 175–177). Male physiology and morphological endpoints were similarly affected by intrauterine position (165, 167, 177–179).

The endocrine milieu of the uterine environment has been implicated in these effects because differences in hormonal exposure have been observed based on intrauterine position (Fig. 2). The production of testosterone in male mice starting at approximately d 12 of gestation allows for passive transfer of this hormone to neighboring fetuses (159, 160, 180). Thus, fetuses positioned between two male neighbors have slightly higher testosterone exposures compared with fetuses positioned between one male and one female or two female neighbors (168, 181–183). These data indicate that very small differences in hormone exposures during fetal development are capable of influencing a variety of endpoints, many of which become apparent only during or after puberty. Furthermore, small differences in hormone exposures may be compounded by other genetic variations such as those normally seen in human populations.

Intrauterine effects have been observed in animals with both large litters and singleton or twin births including ferrets, pigs, hamsters, voles, sheep, cows, and goats (136, 184, 185). But perhaps the most compelling evidence for intrauterine effects comes from human twin studies. Many

**Figure 2.**

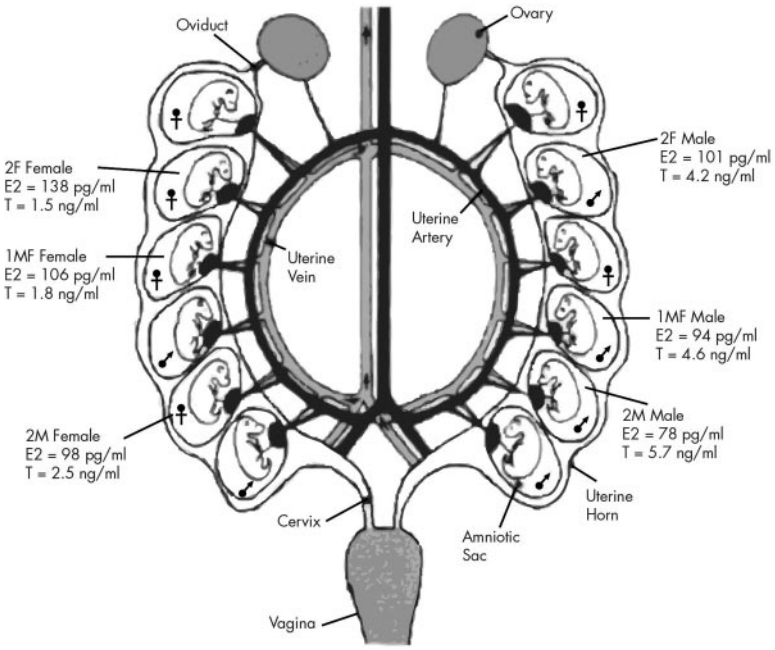



Figure 2. Intrauterine position produces offspring with variable circulating hormone levels. Fetuses are fixed in position in the bicornate rodent uterus, thus delivery via cesarean section has allowed for study of the influence of intrauterine position on behaviors, physiology, and organ morphology. Illustrated here are the differences in estradiol (E2) and testosterone (T) concentrations measured in male and female fetuses positioned between two male neighbors (2M), two female neighbors (2F), or neighbors of each sex (1MF). Direction of blood flow in the uterine artery (dark vessel) and vein (light vessel) is indicated by an *arrow* (159).

studies have found that the sex of the fetuses impacts the phenotype of one or more of the twins, with significant evidence suggesting that male twins strongly influence a female co-twin; endpoints including sensation seeking (186), ear superiority (187, 188), brain and cerebellum volume (189), masculine/feminine behaviors and aggression levels (190–192), handedness (193, 194), reproductive fitness (192, 195), finger length ratios (196), risk for developing eating disorders (197), and birth weight (198) were all affected in females with a male twin. From these studies, many authors have concluded that testosterone from male fetuses influences developmental parameters in female twins; typically, male same-sex twins do not display altered phenotypes for these endpoints. Yet importantly, limited studies indicate that female twins can influence their uterine pairs, with some behaviors affected in male co-twins (191); breast cancer incidence in women and testicular cancer in men have also been shown to be influenced by having a female co-twin (83, 199, 200).

Although the mechanisms for these intrauterine effects are not completely understood, very small differences in hormone exposures have been implicated, making the effects of twin gestations a natural example of low-dose phenomena. In the human fetus, the adrenals produce androgens that are converted to estrogen by the enzyme aromatase, specifically in the placenta. In a human study designed to compare hormone levels in the amniotic fluid, maternal serum, and umbilical cord blood of singleton male and female fetuses, significant differences were observed in the concentrations of testosterone, androstenedione (A4), and estradiol (201). Specifically, amniotic fluid concentrations of testosterone and A4 were approximately twice as high in male fetuses, whereas estradiol concentrations were slightly, but significantly, higher in female fetuses. Yet, interestingly, there were no differences for any of the hormones in maternal serum, similar to findings in mice that litters with a high proportion of males or females did not impact testosterone, estradiol, or progesterone serum levels in mothers (180). In umbilical cord serum, concentrations of A4 and estradiol were higher in males compared with females (201), although it must be noted that these samples were collected at parturition, long after the fetal period of sexual differentiation of the reproductive organs.

Several studies have specifically compared steroid hormone levels in maternal and umbilical cord blood samples collected from same-sex and opposite-sex twins. Male twins, whether their co-twin was a male or a female, had higher blood concentrations of progesterone and testosterone compared with female twins (202). Furthermore, for both sexes, dizygotic twins had higher levels of these hormones, as well as estradiol, compared with monozygotic twins. Fetal sex had no effect on maternal concentrations of testosterone, progesterone, or estrogen, suggesting that any differences observed in fetal samples are due to contributions from the fetuses' own endocrine systems and the placental tissue (203). Yet an additional study conducted in women carrying multiple fetuses (more than three) indicates that both estradiol and progesterone concentrations in maternal plasma increase with the number of fetuses, and when fetal reduction occurs, these hormone levels remain elevated (204).

It has been proposed that low-dose effects seen in different intrauterine positions in litter-bearing animals could be an evolutionary adaptation, whereby the genotypes of the fetuses are relatively similar but a range of phenotypes can be produced via differential hormone exposures (136, 168). For example, female mice positioned between two females are more docile and thus have better

reproductive success when resources are plentiful, but females positioned between two males are more aggressive and therefore are more successful breeders under stressful conditions (161, 171, 175). In this way, a mother produces offspring with variable responses to environmental conditions, increasing the chances that her own genetic material will continue to be passed on. Yet although there is evidence to suggest that a variable intrauterine environment is essential for normal development (171), intrauterine positional effects appear to have little effect on offspring phenotypes in inbred rodent strains (168, 205). This result may be related to the link between genetic diversity and hormone sensitivity (206, 207), suggesting that outbred strains are the most appropriate for studying endocrine endpoints and are also most similar to the effects of low doses of hormones on human fetuses.

Finally, it has been proposed that similar mechanisms are used by the developing fetus in response to natural hormones via intrauterine position and EDCs with hormonal activity (136). To this end, several studies have examined the effects of both exposure to an EDC and intrauterine position or have considered the effect of intrauterine position on the response of animals to these chemicals (174, 176, 181, 208, 209). For example, one study found that intrauterine position affected the morphology of the fetal mammary gland, yet position-specific differences were obliterated by BPA exposure (176). Additional studies suggest that prostate morphology is disrupted by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure in males positioned between two females, but this chemical does not affect prostate morphology in males positioned between two males (181). Finally, male rodents positioned between two males have higher glucose intolerance than males positioned between two females, yet when these males are given a diet high in phytoestrogens, glucose tolerance is dramatically improved in the males positioned between two males, whereas their siblings positioned between two females do not benefit (209). What is clear from these studies is that low doses of natural hormones are capable of altering organ morphology, physiology, and reproductive development, similar to the effects of EDCs.

It has been suggested that the endocrine system allows for homeostatic control and that the aim of the endocrine system is to "maintain normal functions and development in the face of a constantly changing environment" (210). Yet studies from intrauterine position, together with studies of EDCs (see *Sections II.C–F*), clearly indicate that the fetal endocrine system cannot maintain a so-called homeostasis and is instead permanently affected by exposures to low doses of hormones.

## II. Demonstrating Low-Dose Effects Using a WoE Approach

### A. Use of a WoE approach in low-dose EDC studies

In 2001, the NTP acknowledged that there was evidence to support low-dose effects of DES, genistein, methoxychlor, and nonylphenol (2). Specifically, the NTP expert panel found that there was sufficient evidence for low-dose effects of DES on prostate size; genistein on brain sexual dimorphisms, male mammary gland development, and immune responses; methoxychlor on the immune system; and nonylphenol on brain sexual dimorphisms, thymus weight, estrous cyclicity, and immune responses. Using the NTP's definitions of low dose (*i.e.* effects occurring in the range of typical human exposures or occurring at doses lower than those typically used in standard testing protocols), we propose that most if not all EDCs are likely to have low-dose effects. Yet an important caveat of that statement is that low-dose effects are expected for particular endpoints depending on the endocrine activity of the EDC, and not for any/all endocrine-related endpoints. For example, if a chemical blocks the synthesis of a hormone, blood levels of the hormone are expected to decline, and the downstream effects should then be predicted from what is known about the health effects of low hormone levels. In contrast, if a chemical binds a hormone receptor, the effects are expected to be very complex and to be both tissue specific and dose specific. Finally, most EDCs interact with multiple hormone pathways, or even multiple hormone receptors, making the expected effects even more complex and context specific (211–213).

Table 3 summarizes a limited selection of chemicals that have evidence for low-dose effects, with a focus on *in vivo* animal studies. As seen by the results presented in this table, low-dose effects have been observed in chemicals from a number of classes with a wide range of uses including natural and synthetic hormones, insecticides, fungicides, herbicides, plastics, UV protection, and other industrial processes. Furthermore, low-dose effects have been observed in chemicals that target a number of endocrine endpoints including many that act as estrogens and antiandrogens as well as others that affect the metabolism, secretion, or synthesis of a number of hormones. It is also clear from this table that the cutoff for low-dose effects is not only chemical specific but also can be effect dependent. And finally, although this table is by no means comprehensive for all EDCs or even the low-dose effects of any particular chemical, the affected endpoints cover a large range of endocrine targets.

Several EDCs have been well studied, and the number of publications focusing on low-dose effects on a particular developmental endpoint is high; however, other

**TABLE 3.** EDCs with reported low-dose effects in animals (or humans, where stated)

| Chemical | Use | EDC action | Low-dose cutoff | Affected endpoint | Refs. |
|---|---|---|---|---|---|
| Aroclor 1221 (PCB mixture) | Coolants, lubricants, paints, plastics | Mimics estrogens, antiestrogenic activity, *etc.* | 0.1–1 mg/kg (produces human blood levels) | Brain sexual dimorphisms | 683, 684 |
| Atrazine | Herbicide | Increases aromatase expression | 200 $\mu$g/liter (334, 335) | Male sexual differentiation/development | See this review |
| BPA | Plastics, thermal papers, epoxy resins | Binds ER, mER, ERR$\gamma$, PPAR$\gamma$, may weakly bind TH receptor and AR | 400 $\mu$g/kg · d (produces human blood concentrations) | Prostate, mammary gland, brain development and behavior, reproduction, immune system, metabolism | See this review |
| Chlordane | Insecticide | Binds ER | 100 ng/g (produces human blood levels) | Sexually dimorphic behavior | 685 |
| Chlorothalonil | Fungicide, wood protectant | Aromatase inhibitor | 164 $\mu$g/liter (environmental concentrations, EPA) | Corticosterone levels (amphibians) | 686 |
| Chlorpyrifos | Insecticide | Antiandrogenic | 1 mg/kg · d (EPA) | Acetylcholine receptor binding (brain) | 687 |
| DDT | Insecticide | Binds ER | 0.05 mg/kg (EPA) | Neurobehavior | 688 |
| DES | Synthetic hormone | Binds ER | 0.3–1.3 mg/kg · d (dose typically administered to pregnant women) | Prostate weight | 689 |
| Dioxin (TCDD) | Industrial byproduct | Binds AhR | 1 $\mu$g/kg · d (397) | Spermatogenesis, immune function and oxidative stress, tooth and bone development, female reproduction, mammary gland, behavior | See this review |
| Genistein | Phytoestrogen | Binds ER | 50 mg/kg (EPA) | Brain sexual dimorphisms | 690 |
| Heptachlor | Insecticide | Induces testosterone hydroxylases | 0.15 mg/kg · d (EPA) | Immune responses | 691 |
| Hexachlorobenzene | Fungicide | Modulates binding of ligand to TRE, weakly binds AhR | 0.08 mg/kg · d (EPA) | Anxiety and aggressive behaviors | 692 |
| Maneb | Fungicide | Inhibits TSH release, may bind PPAR$\gamma$ | 5 mg/kg · d (EU Commission) | Testosterone release | 693 |
| Methoxychlor | Insecticide | Binds ER | 5 mg/kg · d (WHO) | Immune system | 694, 695 |
| 4-Methylbenzylidine camphor | UV screen | Weakly estrogenic | 10 mg/kg · d (Europa) | Sexual behavior | 696 |
| Methyl paraben | Preservative | Estrogenic | 1000 mg/kg · d (EFSA) | Uterine tissue organization | 697 |
| Nicotine | Natural alkaloid in tobacco | Binds acetylcholine receptors, stimulates epinephrine | Human use of nicotine substitutes | Incidence of cryptorchidism (humans) | 698 |
| Nonylphenol | Detergents | Weakly estrogenic | 15 mg/kg · d (EPA) | Testosterone metabolism | 699 |
| Octylphenol | Rubber bonding, surfactant | Weakly binds ER, RXR, PRGR | 10 mg/kg · d (700) | Testes endpoints | 701 |
| Parathion | Insecticide | | 0.2 mg/kg · d (WHO) | Cognitive and emotional behaviors | 702 |
| PBDE-99 | Flame retardant | Alters TH synthesis | 0.3 mg/kg · d (EPA) | TH levels in blood | 703 |
| PCB180 | Industrial lubricant, coolant | Impairs glutamate pathways, mimics estrogen | Examined normal human populations | Diabetes (humans) | 704 |
| PCB mixtures | Coolants, lubricants, paints, plastics | Binds AhR, mimic estrogens, antiestrogenic activity, etc. | Each at environmentally relevant levels | TH levels | 705 |
| Perchlorate | Fuel, fireworks | Blocks iodide uptake, alters TH | 0.4 mg/kg · d (436) | TSH levels (humans) | See this review |
| Sodium fluoride | Water additive (to prevent dental caries), cleaning agent | Inhibits insulin secretion, PTH, TH | 4 mg/liter water (EPA standard) | Bone mass and strength | 706 |
| Tributyltin oxide | Pesticide, wood preservation | Binds PPAR$\gamma$ | 0.19 mg/kg · d (EPA) | Obesity | 707 |
| Triclosan | Antibacterial agent | Antithyroid effects, androgenic and estrogenic activity | 12 mg/kg · d (Europe SCCP) | Altered uterine responses to ethinyl estradiol | 708 |
| Vinclozolin | Fungicide | Antiandrogenic | 1.2 mg/kg · d (EPA) | Male fertility | 709 |

EDC action indicates that for some chemicals, an effect is observed (*i.e.* estrogenic, androgenic), but for many EDCs, complete details of receptor binding are unavailable or incomplete. Low-dose cutoff means the lowest dose tested in traditional toxicology studies, or doses in the range of human exposure, depending on the data available. Affected endpoint means at least one example of an endpoint that shows significant effects below the low-dose cutoff dose. This list is not comprehensive, and the lack of an endpoint on this table does not suggest that low doses do or do not affect any other endpoints. AR, Androgen receptor; EFSA, European Food Safety Authority; ERR, estrogen related receptor; PCB, polychlorinated biphenyl; PPAR$\gamma$, peroxisome proliferator-activated receptor-$\gamma$; PRGR, progesterone receptor; RXR, retinoid X receptor; SCCP, Scientific Committee on Consumer Products; TH, thyroid hormone; TRE, thyroid response element; WHO, World Health Organization.

chemicals are less well studied with fewer studies pointing to definitive low-dose effects on a given endpoint. In fact, there are a significant number of EDCs for which high-dose toxicology testing has been performed and the no observed adverse effect level (NOAEL) has been derived, but no animal studies in the low-dose range have been conducted, and several hundred additional EDCs where no significant high- or low-dose testing has been performed (see Table 4 for examples). Balancing the large amount of data collected from some well-studied chemicals like BPA and atrazine with the relative paucity of data about other chemicals is a difficult task.

**TABLE 4.** Select examples of EDCs whose potential low-dose effects on animals remain to be studied

| Chemical | Use | EDC action | Low-dose cutoff |
|---|---|---|---|
| Antiseptics and preservatives | | | |
| Butyl paraben | Preservative (cosmetics) | Estrogenic, antiandrogenic | 2 mg/kg · d (EPA) |
| Propyl paraben | Antimicrobial preservative found in pharmaceuticals, foods, cosmetics, and shampoos | Estrogenic activity | LOAEL 10 mg/kg · d, NOEL 6.5 mg/kg · d (Europa) |
| Cosmetics and personal care products | | | |
| 2,4-Dihydroxybenzophenone | UV absorber in polymers, sunscreen agent | Estrogenic activity | Not identified |
| 3-Benzylidene camphor | UV blocker used in personal care products | Estrogenic activity | 0.07 mg/kg · d (710) |
| 4,4′-Dihydroxybenzophenone | UV light stabilizer used in plastics, cosmetics, adhesives, and optical fiber | Estrogenic activity | Not identified |
| Benzophenone-2 | Used in personal care products such as aftershave and fragrances | Estrogenic activity, changes in $T_4$, $T_3$, and TSH levels, alterations in cholesterol profile | NOEL 10–333 mg/kg · d (711) |
| Benzophenone-3 | UV filter | Estrogenic, PPARγ activator | 200 mg/kg · d (Europa) |
| Multiple use (other) | | | |
| Melamine | Flame-retardant additive and rust remover; used to make laminate, textile, and paper resins; metabolite of cyromazine | Affects voltage-gated $K^+$ and $Na^+$ channels and $Ca^{2+}$ concentrations in hippocampal neurons | 63.0 mg/kg · d (FDA) |
| Resorcinol | Used in the manufacturing of cosmetics, dyes, flame retardants, hair dye formulations, pharmaceuticals, skin creams, and tires | Alters $T_4$ and TSH levels | 80.00 mg/kg · d (Europa) |
| Pesticides | | | |
| Aldrin[a] | Insecticide | Estrogenic activity | 0.025 mg/kg · d (Health Canada) |
| Alachlor | Herbicide | Decreases serum $T_4$, binds PR, weakly binds ER | 1 mg/kg · d (EPA) |
| Amitrole | Herbicide | Decreases thyroid hormone | 0.12 mg/kg · d (FAO) |
| Bitertanol | Fungicide | Alters aromatase | 30 mg/kg · d (EPA) |
| Carbendazim | Fungicide | Affects FSH, LH, and testosterone levels; alters spermatogenesis and Sertoli cell morphology | 8 mg/kg · d (712) |
| Diazinon | Insecticide | Alters glucocorticoids | 0.065 mg/kg · d (CDC) |
| Endrin[a] | Insecticide | Stimulates glucocorticoid receptor | 0.025 mg/kg · d (CDC) |
| Fenoxycarb | Insecticide | Alters acetylcholinesterase | 260 mg/kg · d (CDC) |
| Mirex[a] | Insecticide | Decreases testosterone levels | 0.075 mg/kg · d (CDC) |
| Zineb | Fungicide | Alters $T_4$ and dopamine levels | LOAEL 25 mg/kg · d (EPA) |
| Ziram | Fungicide | Alters norepinephrine levels | 1.6 mg/kg · d (EPA) |
| Resins | | | |
| Bisphenol F | Used in polycarbonates | Alters $T_4$, $T_3$, and adiponectin levels, has estrogenic activity | LOAEL 20 mg/kg · d (713) |
| Styrene | Precursor to polystyrene | Alters dopamine | 200 mg/kg · d (EPA) |

PPARγ, peroxisome proliferator-activated receptor-γ; PR, progesterone receptor.

[a] These chemicals were identified in the 1990s as part of the dirty dozen, 12 chemicals that were acknowledged to be the worst chemical offenders because of their persistence in the environment, their ability to accumulate through the food chain, and concerns about adverse effects of exposures to wildlife and humans. These chemicals were banned by the Stockholm convention and slated for virtual elimination. Yet there is still very little known about the low-dose effects of these chemicals, likely in the range of past and current human and/or wildlife exposures.

WoE approaches have been used in a large number of fields to determine whether the strength of many publications viewed as a whole can provide stronger conclusions than any single study examined alone. Although the term, weight of evidence, is used in public policy and the scientific literature, there is surprisingly little consensus about what this term means or how to characterize the concept (214). Historically, risk assessors have used qualitative

approaches (*i.e.* professional judgment to rank the value of different cases) and quantitative approaches (*i.e.* scoring methods to produce statistical and mathematical determinations of chemical safety), but it has been argued that these methods lack transparency and may produce findings that are unrepeatable from one risk assessor to another (215, 216). Whatever the method used, when EDCs are being assessed, it is important to use the principles of endocrinology to establish the criteria for a WoE approach. We do this in *Section II.B*, identifying three key criteria for determining whether a study reporting no effect should be incorporated into a WoE approach. It also should be noted that in epidemiology, the term, weight of evidence, is typically not used, but the concept is actuated by meta-analysis, formally and quantitatively combining data across studies, including a plot of individual and pooled study findings and also a measure of heterogeneity of findings between studies.

For some well-studied chemicals, there are large numbers of studies showing both significant effects, and additional studies showing no effects, from low-dose exposures. In these cases, extensive work is needed to deal with discordant data collected from various sources; studies showing no effect of low-dose exposures must be balanced in some way with those studies that do show effects. As stated by Basketter and colleagues (217), "it is unwise to make a definitive assessment from any single piece of information as no individual assay or other assessment . . . is 100% accurate on every occasion . . . This means that from time to time, one piece of conflicting data has to be set aside." WoE approaches in EDC research have typically dealt with datasets that have some conflicting studies, and these conflicts are even more difficult to sort out when studies have attempted to directly replicate published findings of adverse effects (see for example Refs. 218–221).

Most previously published WoE analyses have examined chemicals broadly (asking questions such as, "Does BPA produce consistent adverse effects on any endpoint?") (see Ref. 222). This can lead to problems including those encountered by the NTP expert panel, which found that there was some evidence for low-dose effects of BPA on certain endpoints but mixed findings for other endpoints. For example, the panel noted that some studies found low-dose effects of BPA on the prostate, but other studies could not replicate these findings. In *Section II.B*, we address criteria that are needed to accept those studies that are unable to detect low-dose effects of chemicals; these criteria were not used by the NTP in 2001, but they are essential to address controversies of this sort and perform WoE analyses using the best available data. In the sections that follow, we employed a WoE approach to examine the evidence for low-dose effects of single chemicals on selected endpoints or tissues, also paying attention to when in development the EDC in question were administered.

### B. Refuting low-dose studies: criteria required for acceptance of studies that find no effect

Over the past decade, a variety of factors have been identified as features that influence the acceptance of low-dose studies (69, 71, 76, 77, 90, 205, 223, 224). In fact, the NTP low-dose panel itself suggested that factors such as strain differences, diet, caging and housing conditions, and seasonal variation can affect the ability to detect low-dose effects in controlled studies (2). In particular, three factors have been identified; when studies are unable to detect low-dose effects, these factors must be considered before coming to the conclusion that no such effects exist.

#### *1. Negative controls confirm that the experimental system is free from contamination*

Although all scientific experiments should include negative (untreated) controls, this treatment category is particularly important for EDC research. When a study fails to detect low-dose effects, the observed response in control animals should be compared with historical untreated controls; if the controls deviate significantly from typical controls in other studies, it may indicate that these animals were, in fact, treated or contaminated in some way or that the endpoint was not appropriately assessed (77, 205, 225). For example, if an experiment was designed to measure the effect of a chemical on uterine weight, and the control uteri have weights that are significantly higher than is normally observed in the same species and strain, these animals may have been inadvertently exposed to an estrogen source, or the uteri may not have been dissected properly by the experimenters. In either case, the study should be examined carefully and likely cannot be used to assess low-dose effects; of course, untreated controls should be monitored constantly because genetic drift and changes in diet and housing conditions can also influence these data, thus explaining changes from historical controls. Importantly, several types of contamination have been identified in studies of EDCs including the leaching of chemicals from caging or other environmental sources (226, 227), the use of pesticide-contaminated control sites for wildlife studies and contaminated controls in laboratory studies (76), and even the use of food that interferes with the effects of EDCs (224, 228). It is also important to note that experiments must consider the solvent used in the administration of their test chemical, and thus good negative controls should test for effects of the solvent itself. Using solvent negative controls helps prevent false posi-

tives as well as the possibility that the vehicle could mask the effects of the chemical being studied.

#### *2. Positive controls indicate that the experimental system is capable of responding to low doses of a chemical acting on the same pathway*

Many studies do not include a positive control, either because of the size and cost of the experiment when including an additional treatment or because an appropriate positive control has not been identified for the endpoint being examined. If the experiment detects an effect of the chemical in question, the exclusion of a positive control does not necessarily affect the interpretation of the results; instead, it can be appropriately concluded that the test chemical is significantly different from unexposed (but similarly handled/treated) negative controls. However, if the study fails to detect low-dose effects of a test chemical, no convincing conclusion can be made; in this case, a positive control is required to demonstrate that the experimental system was capable of detecting such effects (71, 75, 77, 205).

Several issues must be considered when addressing whether the positive control confirms the sensitivity of the assay. First, an appropriate chemical must be selected, and it must be administered via the appropriate route, *i.e.* if the test chemical is administered orally, a positive control that is orally active, such as ethinyl estradiol, should be used; if the test chemical is administered sc, a positive control that is active via this route, such as 17$\beta$-estradiol, is most appropriate. The use of 17$\beta$-estradiol in studies that use oral exposures is particularly inappropriate (see Ref. 229) for example) because this hormone, like most natural steroids, has very low oral activity (77). Second, the positive control chemical must be examined, and effective, at appropriately low doses. Thus, if the test chemical is 100 times less potent than the positive control, a dose of the positive control 100 times lower than the test compound must produce effects (69, 71, 205). For example, studies that report effects of ethinyl estradiol only at doses that are hundreds of times higher than the dose that is effective in contraceptives (230) are not capable of detecting low-dose effects of test chemicals. Without appropriate and concurrent positive and negative controls, studies that fail to detect low-dose effects of test chemicals should be rejected.

#### *3. Species and animal strains that are responsive to EDCs must be used*

The NTP expert panel specifically noted that "because of clear species and strain differences in sensitivity, animal-model selection should be based on responsiveness to endocrine-active agents of concern (*i.e.* responsive to positive controls), not on convenience and familiarity" (2). An analysis of the BPA literature clearly showed that many of the studies that failed to detect effects of low doses used the Charles River Sprague-Dawley rat (75); this strain was specifically bred to have large litters (231), and many generations of inbreeding have rendered the animal relatively insensitive to estrogens (205). The NTP expert panel noted the lack of effects of BPA on Sprague-Dawley rats and concluded that there were clear differences in strain sensitivity to this chemical (2). Importantly, this may not be true for Sprague-Dawley rats that originate from other vendors, indicating that animal origin can also influence EDC testing.

Many studies in mice (138, 206, 207, 232–234) and rats (232, 235–239) have described differences displayed between two (or more) animal strains to a natural hormone or EDC. Often these differences can be traced to whether a strain is inbred or outbred. Genetically diverse strains are generally found to be more sensitive to estrogens (206). Importantly, well-controlled studies demonstrate that strain differences in response to estrogen treatment may be organ dependent or may even differ between levels of tissue organization within the same organ. For example, the Sprague-Dawley rat is more sensitive to ethinyl estradiol than other strains when measured by uterine wet weight. However, when other endpoints were measured, *i.e.* height of cells in the uterine epithelium, the Sprague-Dawley rat was indistinguishable from the DA/Han rat; instead, the Wistar rat had the most heightened response (237). Additionally, there are data to indicate that strain differences for one estrogen may not be applicable for all estrogenic chemicals. In comparing the responses of DA/Han, Sprague-Dawley, and Wistar rats to other xenoestrogens, additional differences were observed including a greater increase in uterine wet weight of DA/Han and Sprague-Dawley rats but not Wistar rats after exposure to 200 mg/kg BPA; increased uterine epithelium thickness was observed in Wistar and Sprague-Dawley rats but not DA/Han rats after exposure to 200 mg/kg octylphenol (237). Attempts have been made, at times successfully, to map the differences in strain response to genetic loci (240). However, it appears that strains with differences in response that manifest in some organs do not have divergent responses in other organs, a phenomenon that is not explained by genetic differences alone. For these reasons, the NTP's recommendation that scientists use animals that are proven responsive to EDCs (2) must be observed.

#### *4. Additional factors?*

Additional factors have also been identified as influential in the ability (or inability) to detect low-dose effects in

EDC studies. Although these factors must be considered when interpreting studies and using a WoE approach, some issues that were previously identified as essential factors in the design of studies (*i.e.* route of administration) have more recently been disputed (241).

The first factor is the use of good laboratory practices (GLP) in the collection of data. When assessing the EDC literature for risk assessment purposes, the FDA and European Food Safety Authority (EFSA) have given special prominence to studies that complied with GLP guidelines, essentially giving scientific priority to industry-funded studies because that group typically conducts GLP guideline studies (33, 242). Because GLP guidelines are designed only to control data collection, standards for animal care, equipment, and facility maintenance, and they do not ensure that studies were designed properly with the appropriate controls, it has been argued that the use of GLP methods is not appropriate or required for EDC studies (69).

GLP studies are typically large, with dozens of animals studied for each endpoint and at each time point. Thus, it has been concluded that these studies are better simply because they are larger. Yet small studies designed with the use of power analysis, statistical tools that allow researchers to determine *a priori* the number of animals needed to determine significant differences based on effect size, are equally capable of detecting effects while reducing the number of animals used (69). GLP studies also typically (but not necessarily) rely upon standardized assays, which are not generally considered contemporary tools and are often shown to be incapable of detecting adverse effects on endpoints that employ modern tools from molecular genetics and related disciplines. Furthermore, some fields of EDC research have no GLP studies (243). Finally, there is no published evaluation of whether studies performed under GLP are more capable of providing accurate results. The priority given to GLP studies therefore does not appear to have been justified based on any comparative analysis. Thus, as long as studies include appropriate measures of quality assurance, they need not be performed under GLP standards to provide reliable and valuable information, and many GLP studies are inadequate to assess important and relevant endpoints. Instead, the most valuable studies consider the factors presented above, along with appropriate dose selections and choice of endpoint.

The second factor worth considering is the source of funding for studies. In several fields, significant controversy has been produced based on the results obtained from independent scientists compared with results obtained from scientists affiliated with the chemical industry (75, 76). Funding source *per se* should not dictate the outcome of a research study, but that does not mean that researchers are not subject to underlying biases. In our own WoE analyses, presented in *Sections II.C–G*, we do not discount studies merely because they were conducted with industry funds, nor do we lend higher weight to studies conducted in independent or government laboratories; if a study, regardless of funding, finds no effect of a chemical, it is given weight only if the three criteria described in *Sections II.B.1–3* (successful and appropriate negative and positive controls and appropriate choice of animal model) were met.

To perform a WoE evaluation, we identified some basic information about the chemical in question, the dose that would be considered a low-dose cutoff, and the studies in support of and against low-dose effects. We then considered whether the majority of studies found effects of low doses of a chemical on a single endpoint in question. If studies did not find low-dose effects, we considered whether they adhered to the criteria discussed above for proper design of an EDC low-dose study. In particular, we considered whether appropriate animal strains as well as positive and negative controls were used. With regard to animal strain, as discussed briefly in *Section II.B.3*, there is variability between animal strains that can significantly influence the ability to detect effects of EDCs; using insensitive strains to produce negative data cannot refute positive data in a sensitive strain. In several cases, it was easy to conclude that there was a strong case for low-dose effects because there were no studies finding no effects at low doses or because all of the negative studies were inappropriately designed. For other chemicals, a significant number of studies found effects on the endpoint being considered, but other (adequately designed) studies refuted those findings. Under those circumstances, we determined whether the findings of harmful effects came from multiple laboratories; when they did, we cautiously concluded that there was evidence for low-dose effects. Below (*Sections II.C–G*), we present five examples where a significant number of studies were available examining low-dose effects of an EDC on a single particular endpoint.

### C. BPA and the prostate: contested effects at low doses?

As discussed briefly above, BPA is one of the best-studied EDCs, with more than 200 published animal studies, many of which focused on low doses (29, 31). The effects of this chemical on wildlife species have also been described in detail (28). BPA is found in a myriad of consumer products, and it leaches from these items under normal conditions of use (4). It has also been regularly detected in air, water, and dust samples. The majority of individuals in industrialized countries have BPA metabolites in their urine, and trends indicate increasing expo-

sures in developing nations like China (87, 244). Although it was long suspected that most human exposures originate from BPA contamination of food and beverages, a study comparing the excretion of BPA metabolites with the length of time spent fasting suggests that there are also likely to be significant exposures from sources other than food and beverages (245). BPA has recently been shown to be used in large quantities in thermal and recycled papers and can enter the skin easily via dermal absorption (246–248). Thus, despite the large amount of information available on BPA sources, our understanding of how these sources contribute to total human exposures remains poor; these studies also point to significant gaps in current knowledge about BPA metabolism in humans (243).

BPA binds to the nuclear and membrane ER, and thus most of the effects of this chemical have been attributed to its estrogenic activity (27). However, there is evidence that it can activate a number of additional pathways, including thyroid hormone receptor, androgen receptor, as well as peroxisome proliferator-activated receptor-$\gamma$ signaling pathways (249–252). The cutoff for a low dose has been set at several different concentrations depending on which studies and definitions are used (see Table 1). The EPA calculated a reference dose for BPA of 50 $\mu$g/kg · d based on a LOAEL of 50 mg/kg · d (38). More recent pharmacokinetic scaling experiments have estimated that exposures to approximately 400 $\mu$g/kg · d produce blood concentrations of unconjugated BPA in the range of human blood concentrations (4). Thus, for the two WoE analyses of the BPA literature we conducted, doses of 400 $\mu$g/kg · d or lower were considered low dose; pharmacokinetic studies from nonhuman primates support the appropriateness of this dose for approximating human exposure levels (253). Furthermore, because this dose is below the toxicological LOAEL, it is a conservative cutoff for low-dose studies (see Refs. 3 and 38 and Table 1).

One of the most well studied and hotly debated examples of a low-dose effect comes from the BPA literature; regulatory agencies and scientists have addressed several times whether low doses of BPA during fetal and perinatal development affect the rodent prostate (118, 205, 254, 255). In 1997, the first study on BPA and the prostate determined that fetal exposure to low doses (2 and 20 $\mu$g/kg · d administered orally to pregnant mice) increased the weight of the adult prostate compared with unexposed male offspring (256). Since that time, several additional studies have verified that prostate weight is affected by fetal exposure to similar low doses (257–259). Studies have also shown that low doses of BPA affect androgen receptor binding activity in the prostate (257), tissue organization, and cytokeratin expression in the gland (260–262) as well as the volume of the prostate and the number and size of dorsolateral prostate ducts (208). Several recent studies have also examined whether low doses of BPA (10 $\mu$g/kg · d) influence the incidence of adult-onset prostatic intraepithelial neoplasia (PIN) lesions. Perinatal BPA exposure, whether administered orally or sc to pups, increases the incidence of PIN lesions in response to a mixture of testosterone and estradiol in adulthood (139, 141, 263); this hormonal cocktail was designed to mimic the endocrine changes associated with aging in men that also typically accompany the onset of prostate cancer. In addition to the effects of BPA on PIN lesions, these low doses also produced permanent alterations in the epigenome of exposed males, with prostates displaying completely unmethylated sequences in genes that are hypermethylated in unexposed controls (140, 263). In examining these studies, although the same effects of BPA on the prostate were not observed in all studies, there is an obvious trend demonstrating that low doses of BPA during early development significantly affect several aspects of prostate development.

Since the initial report showing effects of low doses on the prostate, approximately nine studies, including several designed specifically to replicate the original positive study, have shown no effects of low doses on the prostate (264–272); every one of these studies examined the prostate weight, and Ichihara *et al.* (264) also examined the effects of BPA on PIN lesions (without hormonal treatment) and the response of the prostate to a chemical carcinogen. Three of these studies failed to include a positive control of any kind (264, 268, 270); three studies used DES as a positive control but found no effect from exposure to this potent xenoestrogen (265–267) (*i.e.* the positive control failed); another study used 17$\beta$-estradiol as a positive control, inappropriately administered orally, and found no effects of this hormone on the prostate (271); and two studies used an estrogenic positive control (ethinyl estradiol) and found effects from its exposure, but only at inappropriately high doses (269, 272). These two studies clearly showed that the positive control dose was too high, because rather than increase the weight of the prostate (as seen after low doses of estrogens in other studies), the positive control decreased the weight of the adult prostate (269, 272).

Although this topic was once considered controversial, using a WoE approach, it is clear that there is strong evidence in support of low-dose effects of BPA on the development of the prostate. The evidence clearly shows that several endpoints, including prostate weight, were affected in similar ways in multiple studies from several different labs at doses below 400 $\mu$g/kg · d; most effects were seen at doses below 50 $\mu$g/kg · d. Furthermore, PIN lesions were reported after neonatal exposure to 10 $\mu$g/kg · d with

hormonal treatment in adulthood. No appropriately conducted studies contest this evidence. Therefore, the WoE analysis demonstrates that low doses of BPA significantly alter development of the rodent prostate. The NTP's review of the BPA literature in 2008 indicated that this agency agrees that there is now significant evidence that low-dose BPA adversely affects development of the prostate (273).

### D. BPA and the mammary gland: undisputed evidence for low-dose effects

The mammary gland is a conspicuous choice to examine the effects of estrogenic compounds because this organ depends on estrogen for proper development at several critical periods in life (274). The fetal gland expresses ER in the mesenchymal compartment, and just before birth, the epithelium becomes ER positive as well (275). At puberty, estrogen is responsible for ductal elongation and overall development of the gland, allowing the epithelium to fill the stromal compartment in preparation for pregnancy and lactation. Although BPA is an example of a chemical that has been classified as a weak estrogen because it binds with a much lower affinity to ER$\alpha$ compared with 17$\beta$-estradiol, even weak estrogens are known to affect the development of the mammary gland during early development (276).

In the first study to examine the effects of BPA on the mammary gland, prepubertal rats were exposed to relatively high doses (100 $\mu$g/kg · d or 54 mg/kg · d) for 11 d. After even this short exposure, mammary gland architecture was affected in both dose groups, with increased numbers of epithelial structures and, in particular, structures that suggest advanced development (277). BPA exposure also altered proliferation rates of mammary epithelium and cell cycle kinetics, with an increased number of cells in S-phase and a decreased number of cells in G1. Although relatively high doses of BPA were examined, this initial study indicated that the prepubertal and pubertal gland could be sensitive to BPA.

Many additional studies have examined another critical period, the fetal and neonatal periods, which are sensitive to environmental estrogens (78, 276, 278). Mice exposed prenatally to low doses of BPA via maternal treatment (0.25 $\mu$g/kg · d) displayed altered development of both the stromal and epithelial compartments at embryonic d 18, suggesting that exposures affect tissue organization during the period of exposure (176). In addition, similar low doses produced alterations in tissue organization observed in puberty and throughout adulthood, long after exposures ended, and even induced pregnancy-like phenotypes in virgin females (137, 279–282). Female mice exposed to BPA *in utero* displayed heightened responses to estradiol at puberty, with altered morphology of their glands compared with animals exposed to vehicle *in utero* (138). Another study demonstrated that perinatal BPA exposure altered the mammary gland's response to progesterone (283). Remarkably, all of these effects were observed after maternal exposures to low doses (0.025–250 $\mu$g/kg), suggesting that the gland is extremely sensitive to xenoestrogen exposures. These studies are in contrast to one that examined the effects of higher doses (0.5 and 10 mg/kg · d) when BPA was administered for 4 d to the dam, which reported advanced development of BPA-exposed glands before puberty but no effects in adulthood (284).

Adult exposure to BPA is only now being examined in the mouse mammary gland model. A recent study examined the effects of BPA on mice with mutations in the *BRCA1* gene. This study reported that 4 wks of exposure to a low dose of BPA altered the tissue organization of the mammary gland in ways that are similar to the effects observed after perinatal exposure (285). This study focused on altered development of the gland during exposure; additional studies are needed to determine whether these effects are permanent or whether normal mammary morphology could be achieved by cessation of BPA exposure.

Another obvious endpoint is the effect of BPA exposure on mammary cancer incidence. Several studies indicate that exposure to BPA *in utero* produces preneoplastic (281, 286, 287) and neoplastic lesions (286) in the gland in the absence of any other treatment. Additionally, other studies show that females exposed to BPA during the perinatal period are more sensitive to mammary carcinogens, decreasing tumor latency and increasing tumor incidence (287–290). These studies are also supported by subsequent studies examining gene and protein expression, which show that low-dose BPA specifically up-regulates expression of genes related to immune function, cell proliferation, cytoskeletal function, and estrogen signaling and down-regulates apoptotic genes (282, 288, 289, 291).

Postnatal BPA exposures also influence mammary cancer incidence; animals exposed lactationally to BPA from postnatal d 2 until weaning displayed decreased tumor latency and increased tumor multiplicity after treatment with DMBA [7,12-dimethylbenz(a)anthracene], a carcinogen (292). This study suggested that BPA exposure led to increased cell proliferation and decreased apoptosis in the gland and shifted the period where the gland is most susceptible to mammary carcinogens, a result that has important implications for human breast cancer. Finally, an additional study examined the effects of adult BPA exposure on mammary cancer; this study demonstrated that low doses of BPA accelerate the appearance of mammary tumors in a tumor-prone mouse strain (293). Interestingly,

high doses did not have this effect; thus, this study is also an excellent example of a NMDRC.

Two studies of BPA and the mammary gland seem to contradict this body of literature, but both examined extremely high doses. In the first study, Nikaido *et al.* (294) exposed female mice to 10 mg/kg BPA from postnatal d 15–18. Mammary glands from these animals were examined at 4, 8, and 24 wk of age, and no differences were observed in the exposed animals relative to controls. Although the lack of effects reported in this study could be due to the high dose employed, they could also be related to the relatively short exposure period during the preweaning phase. In the second study, Yin and colleagues (295) examined the effects of BPA during the first few days after birth (0.1 or 10 mg BPA, equivalent to approximately 10 and 1000 mg/kg) on the incidence of mammary tumors after exposure to a mammary carcinogen at puberty. Similar to the study described above, this one also examined the effects of BPA after a relatively short period of exposure (only three injections administered between postnatal d 2 and 6). Although the study showed that BPA affected tissue organization, there was no change in the incidence of tumors in BPA-exposed females. Because both of these studies examined both high doses and relatively short periods of exposure, it is difficult to compare them directly to the studies finding effects of BPA on the mammary gland after longer exposures to lower doses; at the very least, they cannot refute studies suggesting that BPA alters development of this gland.

In summary, the WoE clearly shows that low-dose BPA exposure affects development of the mammary gland, mammary histogenesis, gene and protein expression in the gland, and the development of mammary cancers. In fact, this example of low-dose effects produced remarkably similar effects across more than a dozen studies conducted in several different labs. These results are also consistent with the effects of low-dose BPA exposure on mammary epithelial cells in culture (reviewed in Ref. 30). Although epidemiology studies examining the influence of BPA on breast cancer rates have proven to be inconclusive at best (296), to replicate the animal studies discussed above, epidemiologists must collect information about prenatal and neonatal exposures and relate them to adult breast cancer incidence. These types of studies would take decades to conduct (67) and should take into consideration the effects of other estrogens, because their effects can be additive or even synergistic (143, 144, 297).

Although our analyses of BPA have focused on its effects on the mammary gland and prostate (see *Sections II.C–D*), it is worth noting that several other endpoints have strong data to support the hypothesis that BPA has low-dose effects. In a recent review using similar WoE approaches, Hunt and colleagues (298) focused on those studies that examined the effects of BPA on the oocyte, specifically scrutinizing studies that reported effects, or no effects, on meiotic aneuploidy and other alterations in the intracellular organization and chromosome abnormalities. Similar to what has been observed with the prostate and mammary gland, the effects observed in the oocyte are variable from study to study, but overall consistent, and suggest that BPA exposure produces defects in these cells.

A large number of studies have also focused on the effects of BPA on the brain and behavior, with the most significant effects on sexually dimorphic regions of the brain and behaviors (299–307). Other affected behaviors include social behaviors, learning and anxiety, and maternal-neonate interactions (reviewed in Refs. 29 and 308). The NTP expert panel statement concluded that there were significant trends in these behavioral data and wrote that there was some concern that BPA could have similar effects in humans (273). Low-dose effects have also been reported for BPA in the female reproductive tract (309, 310), immune system (311, 312), maintenance of body weight and metabolism (313, 314), fertility (315–317), and the male reproductive tract (259, 318) (see Refs. 29 and 319 for comprehensive reviews).

### E. Another controversial low-dose example: atrazine and amphibian sexual development

Atrazine is an herbicide that is applied in large volumes to crops, and there is concern that agricultural runoff of this chemical can affect nontarget animal species, especially amphibians that live and reproduce in small ponds and streams where significant amounts of atrazine have been regularly measured (320–322). It is the most commonly detected pesticide in ground and drinking water. Atrazine induces aromatase expression in cells and animals after exposure (323); this ultimately causes an increase in the conversion of testosterone to estrogen (324, 325). This effect has been reported in all vertebrate classes examined: fish, amphibians, reptiles, birds, and mammals, including human cell lines (see Ref. 326 for review). Another well-documented effect of atrazine is that it decreases androgen synthesis and activity, again, in every vertebrate class examined (326). In addition, endocrine-disrupting effects of atrazine occur through a number of other mechanisms, including antiestrogenic activity (327), altered prolactin release (328), and increased glucocorticoid release from the adrenal glands (329, 330), among others (327).

Because of atrazine's indirect effect on estrogen levels, one relevant endpoint that has been given attention is the effect of this chemical on gonad differentiation in various amphibian species. The early gonad is bipotential, and in

mammals, the expression of genes on the Y-chromosome is needed to masculinize the undifferentiated gonad; when this does not occur, the gonad develops into ovarian tissue. In *Xenopus laevis* frogs (and some other animals like birds), the opposite is true: females are heterogametic (*i.e.* ZW-chromosomes) and males have two of the same chromosomes (*i.e.* ZZ). In *X. laevis*, the W-chromosome is the dominant one, containing a gene, DM-W, which induces aromatase expression (331). Thus, having a W-chromosome is needed to produce estrogen; without the conversion of testosterone to estrogen, the frog develops as a male (332). Changes in sex ratio and gonadal morphology are therefore good indicators that an estrogen, or a chemical that up-regulates aromatase and indirectly increases estrogen levels, is present (76).

Determining a low-dose cutoff for atrazine is not a simple task. Although the safe limit of 3 $\mu$g/liter in drinking water was set by the EPA, actual levels in the environment often exceed this concentration (333), and levels in ponds and streams can reach 100 $\mu$g/liter (322) or more. In traditional toxicology studies examining several amphibian species, the LOAEL was set at 1.1 mg/liter, and the no observed effect level (NOEL) was 200 $\mu$g/liter (334, 335). Thus, using the definitions of low dose established by the NTP (2), we consider any treatment at or below 200 $\mu$g/liter to be a low dose.

In 2002, one of the first published studies to connect atrazine exposures to altered gonadal morphology examined *X. laevis* frogs exposed to 0.01–200 $\mu$g/liter throughout larval development (336). All doses from 0.1–200 $\mu$g/liter produced gonadal malformations including the presence of multiple gonads and hermaphroditism. Several other reports showed similar effects of low doses on gonadal phenotypes including studies that report the production of hermaphrodites and intersex frogs, males with ovotestes, and males with testicular oocytes (337–343). Additional studies showed that low-dose atrazine exposure (0.1–200 $\mu$g/liter in the water) during sexual differentiation caused testicular dysgenesis, testicular resorption, and testicular aplasia in male frogs (343, 344), and others indicated effects on sex ratios (339, 342, 345, 346). Importantly, these effects were not all observed at the same atrazine concentration, and the studies were conducted in several different species, with some reporting effects at low doses but no effects at higher doses (341) and others reporting effects in some but not all species (339). Examining these studies as a whole, there is clearly a pattern of effects that are reproducible from study to study, and they collectively support the hypothesis that atrazine disrupts sex hormone concentrations.

To date, five peer-reviewed studies have reported no effects of atrazine on sex ratios, gonadal morphology, the incidence of testicular abnormalities or testicular oocytes, gonad size, or the incidence of intersex phenotypes (347–351). Little can be ascertained from these negative studies, however, because four did not include any positive control, suggesting that the frogs used in those studies may have been incapable of responding to atrazine or any other hormonal treatment (347–350). Additionally, one of those studies reported testicular oocytes in the control frogs, suggesting either that the negative control population was contaminated with atrazine (or another EDC or hormone), or that an inappropriate strain of *X. laevis* was selected for the experiments (347). Only one study remains that did not find any effects of atrazine; this study used an appropriate positive control (17$\beta$-estradiol) and found effects of that hormone on sex ratios and the incidence of intersex gonads (351). An EPA expert panel noted, however, that this study used a strain of *X. laevis* that was obtained from a new, unexamined population of frogs from Chile and suggested that this strain may be insensitive to environmental chemicals. Furthermore, the panel called for additional analysis of the data in this study, including the statistical approaches; they suggested that an independent laboratory should evaluate the histopathological results; and they requested that atrazine metabolites be measured (352). The panel also proposed that these experiments should be repeated with an established *X. laevis* strain. Taking together the results of those studies that found effects of atrazine on sexual differentiation, and this one negative study, the WoE for the case of low-dose atrazine on sexual differentiation is clearly in support of adverse effects of this chemical.

Just as epidemiological studies have found links between EDCs and human diseases, ecological field studies have examined whether exposure to atrazine in natural environments affects the development of wild amphibians (343, 353–358). These studies have many of the same constraints as those observed in epidemiology: a paucity of data on early life exposures (including exposure levels of controls), limitations on the total number of EDCs that can be measured in environmental and biological samples, and a lack of causative relationships that can be established between exposures and effects. For these reasons, studies that found relationships between atrazine exposure (or concentrations in environmental samples) and effects on one or more aspect of sexual differentiation (343, 353–355) are considered weak, but significant, evidence for low-dose effects. The presence of several studies suggesting a relationship between low-dose exposure to atrazine in the wild and altered sexual differentiation indicates a plausible causal relationship. Because the ecological and laboratory data show similar effects of atrazine on go-

nadal development, this strengthens the conclusions of our WoE that low doses of atrazine cause harm to amphibians.

Feminization of males after atrazine exposure is not restricted to amphibians; exposure of zebrafish to low doses increased the ratio of female to male fish and increased expression of aromatase (359). Close to a dozen additional studies also report that environmentally relevant doses of atrazine can up-regulate aromatase, decrease testosterone, and/or increase estrogen levels in a large number of species (reviewed in Ref. 119), suggesting that low-dose effects of atrazine may be more widespread than their effects on the gonads of amphibians. Other studies indicate that low-dose atrazine affects the immune system and stress responses of salamanders (360–362), survivorship patterns of several frog species (363), and thyroid hormone and plasma ion concentrations in salmon (364).

An important factor to consider when examining the effects of atrazine on different animal models is the difficulty in identifying an appropriate low, environmentally relevant dose for all species. Aquatic animals can be housed in water containing levels of atrazine found in wild habitats, yet no toxicokinetic studies are available to determine what administered dose produces the levels of atrazine metabolites, typically in the parts-per-million or ppb range (365, 366), measured in human samples. There are also no blood or urine measurements in exposed rodents to compare with human levels; thus, extrapolations across species are estimates at best.

Keeping this qualification in mind, exposures in the range of 25–100 mg/kg · d during development have been shown to alter mammary gland development (367, 368), estrous cyclicity (369), serum and intratesticular testosterone concentrations (370), timing of puberty in males and prostate weight (371), and immune function (372) in rodents. Lower doses of atrazine metabolites (0.09–8.73 mg/kg · d) altered development of the mammary gland (373), male pubertal timing and prostate development (374). Identifying the range of doses administered to animals that produce the levels of atrazine and its metabolites measured in human blood and urine is an essential research need to pursue low-dose studies in rodents and other mammals.

### F. Dioxin and spermatogenesis: low-dose effects from the most potent endocrine disruptor?

Dioxin, or TCDD, is formed as a byproduct of industrial processes as well as during waste incineration. Because TCDD is extremely toxic to some animals, with 1 $\mu$g/kg capable of killing 50% of guinea pigs, it has been labeled the most toxic chemical on earth (375). But interestingly, other animals are less sensitive to lethal effects of TCDD, with an $LD_{50}$ of approximately 1000 $\mu$g/kg in hamsters, and studies also suggest that humans are not a hypersensitive species for lethality (376). Additionally, there are differences in the half-life of TCDD in different animals; in rodents, the half-life is 2–4 wks, but in humans, the half-life is approximately 10 yrs, and additional factors influence TCDD pharmacokinetics including the exposure level and the amount of body fat present (377–379). In cell cultures, doses as low as $10^{-11}$ M are toxic, with decreased viability observed even in cells maintained in nonproliferative states (380).

TCDD binds to the aryl hydrocarbon receptor (AhR), and differences in the affinity for the receptor may be responsible for differences in sensitivity between species (381). The $K_d$ (dissociation constant for receptor-ligand binding kinetics) in human samples typically ranges from 3–15 nM, but in samples from rodents, the $K_d$ is less than 1 nM (382). Importantly, there are also nongenomic pathways affected by TCDD that are mediated by AhR that are typically altered within minutes of TCDD exposure and therefore without changes in transcription (383). Yet many studies suggest that important differences exist between species regarding binding affinity of TCDD for AhR and the toxicity of this chemical, but that other adverse effects, including those related to the endocrine-disrupting activities of TCDD, occur at similar doses (or body burdens) across animal species (384, 385). Thus, it is plausible that AhR affinity alone can predict some, but not all, effects of TCDD and related chemicals.

The mechanisms responsible for many of the endocrine-disrupting activities of TCDD are currently not well understood. Knocking out AhR disrupts morphogenesis of several organ systems even in the absence of a ligand like TCDD, suggesting that this receptor plays important roles in early development (386). AhR is translocated to the nucleus after loss of cell-cell contacts and is often localized to the nucleus in embryonic cells, suggesting that it could have ligand-independent effects on development and/or that endogenous ligands could be present during early development (387). When TCDD is present, AhR translocates to the nucleus and dimerizes with ARNT, the aromatic hydrocarbon receptor nuclear translocator (388). Although the (currently unidentified) physiological activators of AhR are likely to induce rapid on/off signaling via AhR, TCDD and related compounds appear to maintain activation of AhR, and the presence of TCDD prevents the normal action of the AhR signaling pathway in the maintenance of homeostasis (389). This induces changes in the expression of genes and promotes the production of toxic metabolites. These effects may be responsible for some of the endocrine-related endpoints affected by TCDD exposure. Additionally, recent studies have shown complex and intricate interactions between the

AhR and ER signaling pathways (390), suggesting that dioxin may also have indirect effects on some ER-mediated endpoints via AhR signaling.

Teratogenic effects of TCDD have been well documented after high-dose (391, 392) and low-dose exposures (393). These studies show that almost every organ and system in the body is affected by this chemical. High doses that did not produce lethality caused severe weight loss, intestinal hemorrhaging, alopecia, chloracne, edemas, and severe liver damage. Sadly, there are now several examples in humans of accidental exposures after the industrial release of TCDD where a number of individuals have been exposed to large doses (389, 394) as well as a few documented intentional poisonings (395). The tolerated daily intake level was set at 1–4 pg/kg · d, although the doses consumed by nursing infants are likely to exceed these levels by a factor of 10 (375). Adult exposures usually result from the consumption of contaminated foods, and because TCDD is lipophilic, it is concentrated in the fat component of breast milk and therefore passed in large quantities from a nursing mother to her infant.

Using classical toxicology methods, the effects of single TCDD doses were examined in adult male rats, specifically focusing on the effects of this chemical on the number of spermatids per testis and the integrity of the testicular germinal epithelium (396). In one of the earliest studies, Chahoud and colleagues (397) determined a LOAEL of 3 $\mu$g/kg · d and set the NOAEL at 1 $\mu$g/kg · d for effects on the testes. Because there are significant differences in the toxicity of TCDD between animal models, and different endpoints have different identified NOAELs, we have selected the 1 $\mu$g/kg · d identified by Chahoud *et al.* as the cutoff for low-dose studies of this compound. This cutoff is based on the NTP's definition of low dose as occurring at doses lower than those tested in traditional toxicology assessments (2). However, it is important to acknowledge that body burdens that mimic those observed in human populations are likely the best indicators of low doses for TCDD (384), and thus we recommend that future studies determine body burdens after administration of TCDD for the specific strain, origin, and species of animal being tested to ensure that truly low doses, relevant to human populations, are being tested.

Several recent epidemiological studies have indicated that relatively high exposures to TCDD during early life (due to industrial release of high amounts of the chemical) can permanently affect semen quality and sperm count in men (398). Yet epidemiology studies also clearly show that the timing of TCDD exposure can vastly influence the effect of this chemical on spermatogenesis; exposures during perinatal life significantly reduced sperm parameters, but exposures during puberty increased sperm counts; exposures in adulthood had no effect on sperm parameters (399). Thus, it is also important for animal studies to focus on exposures during critical periods for development of the male reproductive tract and spermatogenesis in particular.

We are aware of 18 studies that have examined the effects of low doses ($\leq$1 $\mu$g/kg · d) of TCDD during perinatal development on male fertility endpoints in adulthood. The endpoints assessed vary, including epididymal sperm counts, ejaculated sperm number, daily sperm production, sperm transit rate, and percent abnormal sperm, and the sensitivity of these endpoints appears to impact the ability to detect low-dose effects in different studies (400, 401) (Table 5). In total, 16 rodent studies examined the effect of low-dose TCDD on epididymal sperm count; 12 showed significant effects on this endpoint (402–413), whereas the other four did not (414–417). Of the five studies that examined ejaculated sperm counts, four studies (404, 405, 408), including one examining rhesus monkeys (418), showed effects of low-dose TCDD, *i.e.* a significant decrease in sperm counts; one study found no effect (417). Daily sperm production was a less-sensitive endpoint, with four studies showing significant decreases after prenatal exposure to low doses (402, 403, 407, 409) and four studies showing no effects (406, 412, 413, 416); sperm transit rate was examined in only two studies, although both showed significant decreases in sperm tranfer rates (403, 410); and finally, three studies determined that low-dose TCDD produced abnormalities in sperm appearance or motility (414, 415, 419), but one study was not able to replicate these findings (417).

When examining the TCDD literature as a whole, the WoE strongly suggests that prenatal exposure to low doses of TCDD affects sperm-related endpoints in adulthood (Table 5). In all, only two studies were unable to detect any effect of TCDD on the sperm endpoints assessed, although both studies found effects of TCDD on other endpoints including the weight of the adult prostate (416) and the timing of puberty (417). No study on TCDD used a positive control, likely due to a paucity of information on the mechanisms of dioxin action, but this raises obvious questions about the ability of these experimental systems to detect effects on spermatogenesis. Finally, some of the inability to detect effects of TCDD could be due to the use of insensitive strains, because 1000-fold differences in sensitivity have been reported for different rodent strains (420).

Even though we have focused the majority of our attention on the effects of low-dose TCDD exposure on spermatogenesis, it should be noted that low doses of this chemical affect a multitude of endpoints in animals, altering immune function (421, 422), indicators of oxidative

**TABLE 5.** Summary of low-dose animal studies examining the effects of TCDD on spermatogenesis endpoints

| Study | Administered dose (time of administration) | Animal | Epididymal sperm count | Ejaculated sperm no. | Daily sperm production | Sperm transit rate | % abnormal sperm |
|---|---|---|---|---|---|---|---|
| Mably *et al.* (409) | 0.064–1 μg/kg (gestational d 15) | Rat | Decreased | NA | Decreased | NA | NA |
| Bjerke and Peterson (402) | 1 μg/kg (gestational d 15) | Rat | Decreased | NA | Decreased | NA | NA |
| Gray *et al.* (404) | 1 μg/kg (gestational d 8) | Rat | Not significant | Decreased | NA | NA | NA |
| | 1 μg/kg (gestational d 15) | Rat | Decreased | Decreased | NA | NA | NA |
| | 1 μg/kg (gestational d 11) | Hamster | Decreased | Decreased | NA | NA | NA |
| Sommer *et al.* (408) | 1 μg/kg (gestational d 15) | Rat | Decreased | Decreased | Decreased | Not significant | Not significant |
| Wilker *et al.* (410) | 0.5, 1 or 2 μg/kg (gestational d 15) | Rat | Decreased | NA | Unaffected | Increased | NA |
| Gray *et al.* (405) | 0.05–1 μg/kg (gestational d 15) | Rat | Decreased | Decreased | Decreased | NA | NA |
| Faqi *et al.* (403) | 0.025–0.3 μg/kg (before mating, then 0.005–0.06 μg/kg weekly [to dams]) | Rat | Decreased | NA | Decreased | Increased | Increased |
| Loeffler and Peterson (412) | 0.25 μg/kg (gestational d 15) | Rat | Decreased | NA | Unaffected | NA | NA |
| Ohsako *et al.* (416) | 0.0125–0.8 μg/kg (gestational d 15) | Rat | Not significant | NA | Unaffected | NA | NA |
| Ohsako *et al.* (406) | 1 μg/kg (gestational d 15) | Rat | Decreased | NA | Unaffected | NA | NA |
| | 1 μg/kg/gestational d 18 | Rat | Unaffected | NA | Unaffected | NA | NA |
| | 1 μg/kg/postnatal d 2 (to pups) | Rat | Unaffected | NA | Unaffected | NA | NA |
| Simanainen *et al.* (407) | 0.03–1 μg/kg (gestational d 15) | Rat | Decreased | NA | Decreased | NA | NA |
| Yonemoto *et al.* (417) | 0.0125–0.8 μg/kg (gestational d 15) | Rat | Unaffected | Unaffected | NA | NA | Unaffected |
| Yamano *et al.* (714) | 0.3 or 1 μg/kg (postnatal d 1 and then every week [to dams]) | Rat | Not significant | NA | NA | NA | NA |
| Ikeda *et al.* (715) | 0.4 μg/kg (before mating, then 0.08 μg/kg weekly [to dams]) | Rat | Unaffected | NA | NA | NA | NA |
| Bell *et al.* (414) | 0.05–1 μg/kg (gestational d 15) | Rat | Increased (at certain ages) | NA | NA | NA | Increased |
| Bell *et al.* (415) | 0.0024–0.046 μg/kg (d 12 weeks before pregnancy through parturition) | Rat | Unaffected | NA | NA | NA | Increased |
| Arima *et al.* (418) | 0.03 or 0.3 μg/kg (gestational d 20, then 5% of dose monthly [to dams]) | Rhesus monkey | Decreased | Not significant | NA | NA | Not significant |
| Yamano *et al.* (419) | 0.3 or 1 μg/kg (weekly to dams then pups [all postnatal]) | Rat | NA | NA | NA | NA | Increased |
| Jin *et al.* (411) | 1 μg/kg · d (postnatal days 1–4 [to dams]) | Mouse | Decreased | NA | NA | NA | NA |
| Rebourcet *et al.* (413) | 0.01–0.2 μg/kg (gestational d 15) | Rat | Decreased (at some ages) | NA | Not significant | NA | NA |

Not significant indicates trend for effect but did not reach statistical significance. Unaffected means assessed, but no differences were observed relative to controls. Here, low doses were considered any at or below 1 μg/kg · d (see text for discussion of how this cutoff was established for rodent studies). NA, Not assessed.

stress (423–425), bone and tooth development (426, 427), female reproduction and timing of puberty (428–430), mammary gland development and suceptibility to cancers (431), behaviors (432, 433), and others. In several cases, lower doses were more effective at altering these endpoints than higher ones (423, 424, 426, 433). Epidemiology studies of nonoccupationally exposed individuals also indicate that serum TCDD levels may be linked to diseases in humans as well (434). Mean serum TCDD levels have decreased by a factor of 7 over a 25-yr period (1972–97) in several industrial nations (435), but results from both animal and epidemiological studies suggest that even the low levels detected now could have adverse effects on health-related endpoints.

### G. Perchlorate and thyroid: low-dose effects in humans?

A significant challenge with observing low-dose effects of EDCs in the human population is that human chemical exposures are multivariate along the vectors of time, space, and sensitivities. In addition, chemicals can exert effects on several systems simultaneously. Therefore, associations in human studies between exposures and disease are difficult to reconcile with experimental studies in animal model systems. For this reason, the literature describing the potential impacts of perchlorate contamination on the human population is potentially clarifying because to the best of our knowledge, perchlorate exerts only a single effect, and the pharmacology of perchlorate exposures has been studied in human volunteers (436). This

literature offers a unique perspective into the issue of low-dose effects, perhaps providing important hypotheses to explain mechanistically why high-dose, short-term experiments can fail to predict the outcome of low-dose, lifetime exposures.

In the 2001–2002 NHANES dataset, perchlorate was detected in the urine of each of the 2820 samples tested (437). This widespread exposure means that the human population is being continuously exposed because perchlorate has a half-life in the human body of about 8 h (438). Human exposures to perchlorate are likely attributed to both contaminated drinking water and food (439); in fact, a recent analysis concludes that the majority of human exposure to perchlorate comes from food (440).

The predominant theory proposed to explain the source of perchlorate contamination in the United States is that it has been employed for many decades as the principal oxidant in explosives and solid rocket fuels (441). Perchlorate is chemically stable when wet and persists for long periods in geological systems and in ground water. Because of disposal practices during the 1960s through 1990s, perchlorate became a common contaminant of ground water in the United States (441, 442). Perchlorate is also formed under certain kinds of natural conditions (443), although the relative contributions to human exposure of these different sources is not completely understood. As a result of perchlorate contamination of natural waters, the food supply has become contaminated through irrigation in part because both aquatic and terrestrial plants can concentrate perchlorate more than 100-fold over water levels (444).

This exposure profile in the human population is important because high doses of perchlorate are known to reduce functioning of the thyroid gland, and poor thyroid function is an important cause of developmental deficits and adult disease (445). The primary question is: at what dose does perchlorate inhibit thyroid function sufficiently to cause disease? The current literature, reviewed below, supports the view that background exposure may affect thyroid function in adult women. These exposure levels, however, are considerably lower than predicted by early toxicology experiments in humans.

Perchlorate reduces thyroid function by inhibiting iodide uptake by the sodium/iodide symporter (NIS) (446), which is the only known effect of perchlorate on human physiology (438). NIS is responsible for transporting iodide into the thyroid gland, which is required for the production of thyroid hormone (447). However, NIS is also expressed in the gut (448, 449), in lactating breast (448, 450, 451), and in placenta (452), presumably all as a delivery mechanism for iodide to the developing and adult thyroid gland. Because the NIS transports perchlorate (450), the pathway by which humans take up and concentrate perchlorate is the same as the pathway by which humans take up and concentrate iodide. Interestingly, NIS expression in the human fetal thyroid gland is the rate-limiting step in production of thyroid hormone (453). Moreover, NIS transport of perchlorate explains why high levels of perchlorate are found in human amniotic fluid (454, 455) and breast milk (456–459).

This effect of perchlorate on thyroid function is important because thyroid hormone is essential for normal brain development, body growth as well as for adult physiology (445, 460). Moreover, it has become clear that even small deficits in circulating thyroid hormone in pregnant women (461, 462) or neonates (463) have permanent adverse outcomes. In fact, recent work indicates that very subtle thyroid hormone insufficiency in pregnant women is associated with cognitive deficits in their children (461). Because of the importance of thyroid hormone in development and adult physiology, and because perchlorate is a potent inhibitor of iodide uptake and thyroid hormone synthesis, identifying the dose at which these events occur is critical.

Perchlorate was used medically to reduce circulating levels of thyroid hormone in patients with an overactive thyroid gland in the 1950s and 1960s (reviewed in Ref. 446); therefore, it was reasonable to examine the dose-response characteristics of perchlorate on the human thyroid gland. Because perchlorate inhibits iodide uptake, several studies were performed to evaluate the effect of perchlorate exposure on iodide uptake inhibition in human volunteers (438, 464–466). In one study, 0.5 or 3 mg/d (approximately 0.007 and 0.04 mg/kg · d) perchlorate was administered to healthy volunteers (n = 9 females and 5 males, age 25–65 yr), and no effects were observed (466). Of course, it is important to note that the 2 wk of administration tested in this study is not sufficient to see any effect on serum concentrations of $T_4$ or TSH; the healthy thyroid can store several months' worth of thyroid hormone in the gland (467). Another small study also found no effects of administering 3 mg/d (approximately 0.04 mg/kg · d) on any thyroid endpoint assessed (n = 8 adult males) (464).

In contrast, two studies examining adult volunteers administered perchlorate found effects of this chemical on at least one endpoint. The first found that radioactive iodide uptake was affected by 2 wk of exposure to 10 mg/d (0.13 mg/kg · d), but other measures of thyroid function were not altered (n = 10 males) (465). The second examined adults (n = 37) given doses ranging from 0.007–0.5 mg/kg · d; all but the lowest dose altered radioactive iodide uptake, and only the highest dose altered TSH levels (438). These studies were interpreted to suggest that adults would have to consume 2 liters of drinking water daily that

was contaminated with at least 200 ppb (200 $\mu$g/liter) perchlorate to reach a level in which iodide uptake would begin to be inhibited. Yet, these administered doses are high and relatively acute, so the derivation of a safe dose from these studies, applied to vulnerable populations such as those with low iodide intake, has been strongly disputed (471).

Studies of occupational exposures have also been used to examine the effects of exposure to relatively high levels of perchlorate. In the first such study, more than 130 employees were separated into eight groups based on exposure estimates from airborne perchlorate in the workplace (472). The authors found that individuals with longer daily exposures to perchlorate, due to longer work shifts, had significant decreases in TSH levels compared with individuals with shorter exposures. But this study was hampered because actual exposure levels were not measured via urine or blood samples. A second study examined 37 employees exposed to perchlorate and 21 control employees from an azide factory; actual exposure measures were not conducted, but estimates were calculated based on exposures to perchlorate dust and air samples (473). This study found no effects of perchlorate exposures on any thyroid endpoint, although the sample size examined was small. In the final occupational exposure study, serum perchlorate levels were measured and compared with several measures of thyroid function in workers (n = 29) who had spent several years as employees in a perchlorate production plant (474). In this study, the most complete because of the biomonitoring aspect of the exposure measures, higher perchlorate levels were associated with lower radioactive iodide uptake, higher urinary iodide excretion, and higher thyroid hormone concentrations.

Although iodide uptake was often inhibited in these studies, serum thyroid hormones were typically not altered, perhaps because of sufficient stored hormone. Based on these observations, the National Academy Committee to Assess the Health Implications of Perchlorate Ingestion (467) estimated that perchlorate would have to inhibit thyroid iodide uptake by about 75% for several months to cause a reduction in serum thyroid hormones. Moreover, the drinking water concentration of perchlorate required for this kind of inhibition was estimated to be over 1,000 ppb (438). Therefore, the National Academy of Sciences committee recommended a reference dose of 0.0007 mg/kg · d (467), based on the dose at which perchlorate could inhibit iodide uptake, and the EPA used this value to set a provisional drinking water standard of 15 ppb.

Considering these data and general knowledge about the thyroid system, it was unexpected that Blount *et al.* (475) would identify a positive association between urinary iodide and serum TSH in adult women in the NHANES 2001–2002 dataset. Yet several features of this dataset were consistent with a causal action of perchlorate on thyroid function. First, in the general population of adult women, urinary perchlorate was positively associated with serum TSH. In the population of adult women who also had low urinary iodide, however, urinary perchlorate was more strongly associated with serum TSH and was negatively associated with serum $T_4$. The strength of this association was such that the authors calculated that women at the 50th percentile of perchlorate exposure experienced a 1 $\mu$g/dl $T_4$ reduction (reference range = 5–12 $\mu$g/dl). Should this magnitude of reduction in serum $T_4$ occur in a neonate, measurable cognitive deficits would also be present (476). Finally, Steinmaus *et al.* (477), using the same NHANES dataset, showed that women with low urinary iodide who smoke had an even stronger association between urinary perchlorate and measures of thyroid function. Tobacco smoke delivers thiocyanates, which also inhibit NIS-mediated iodide uptake (446).

The NHANES dataset suggests that perchlorate exposures of 0.2–0.4 $\mu$g/kg · d (440) are associated with depressed thyroid function, even when urinary iodide is not reduced. This is a considerably lower dose than the 7 $\mu$g/kg · d dose required to suppress iodide uptake in the Greer *et al.* (438) study or the 500 $\mu$g/kg · d the NAS estimated would be required for several months to actually cause a decline in serum $T_4$. Therefore, it is reasonable to question whether these associations represent a causative relationship between perchlorate and thyroid function.

A number of epidemiological studies have been published to test for a relationship between perchlorate exposure and thyroid function. Early work used neonatal screening data for $T_4$ as a measure of thyroid function, and the city of birth (Las Vegas, NV, compared with Reno, NV) as a proxy measure of exposure (478, 479). The reported findings were negative, but we now know that all Americans are exposed to perchlorate, so there was considerable misclassification of exposure, and no relationship should have been observed. Several additional studies using similar flawed designs also found no relationship between proxy measures of perchlorate exposures and clinical outcomes (480–484).

A recent study of the neonatal screening data from 1998 in California identified a strong association between neonatal TSH and whether or not the mother resided in a contaminated area (485). This study included over 497,000 TSH measurements and 800 perchlorate measurements. In addition, they used as a cutoff a variety of TSH levels (as opposed to the 99.9th percentile used for the diagnosis of congenital hypothy-

roidism), indicating that perchlorate exposure is not associated with congenital hypothyroidism. Two additional studies have shown similar relationships between perchlorate and TSH levels, particularly in families with a history of thyroid disease (486, 487).

Several studies in pregnant women have failed to identify a relationship between perchlorate exposure and measures of thyroid function (488–490). Although these are important studies that need to be carefully scrutinized, they do not replicate or refute the NHANES dataset. It thus remains important to conduct additional studies exploring the relationship between background exposure to perchlorate and thyroid function in adults, pregnant women, neonates, and infants. This effort will be challenging because of the different characteristics of thyroid function and hormone action at different life stages (460). In addition, it will be important to obtain individual measurements of exposures to perchlorate and other NIS inhibitors (thiocyanate and nitrate), and iodide itself as well as individual measures of thyroid function (free and total $T_4$ and TSH).

If background levels of perchlorate affect thyroid function in any segment of the population, it will be challenging to explain how the high-dose, short-term experiments of Greer *et al.* (438) completely underestimated the sensitivity of the human thyroid gland to perchlorate exposure. One possibility is that physiological systems respond to short durations of robust stress with compensatory mechanisms that reset during periods of long-term stress.

When these data are examined together, several important issues are raised. First, this example illustrates the difficulties inherent in studying human populations; epidemiology yields associations, not cause-effect relationships, in many cases using surrogate markers for perchlorate, and is not able to distinguish short- *vs.* long-term exposure duration. Second, our WoE analysis suggests that there is weak evidence for low-dose effects of perchlorate; further research is needed. The relationship between low-dose perchlorate exposures and thyroid endpoints would be strengthened by the addition of studies that measure biological concentrations of perchlorate and compare them with thyroid endpoints in neonates and other vulnerable populations. Third, the published studies that reported low-dose effects of perchlorate typically examined very specific populations, with several focusing on women with low iodine intake. This observation suggests that some groups may be more vulnerable to low doses of perchlorate than others (491).

### H. Low-dose summary

These examples, and the examples of low-dose effects in less well-studied chemicals (Table 3), provide evidence that low-dose effects are common in EDC research and may be the default expectation for all chemicals with endocrine activity. Many known EDCs have not been examined for low-dose effects, but we predict that these chemicals will have effects at low doses if studied appropriately. Although studies unable to detect effects at low doses have received attention, including some studies designed to replicate others that reported low-dose effects, the majority of these studies contain at least one major design flaw. Thus, a WoE approach clearly indicates that low-dose effects are present across a wide span of chemical classes and activities.

## III. Nonmonotonicity in EDC Studies

A concept related to low dose is that of nonmonotonicity. As noted in *Section I.B*, in a monotonic response, the observed effects may be linear or nonlinear, but the slope does not change sign (Fig. 3, A and B). In contrast, a dose-response curve is nonmonotonic when the slope of the curve changes sign somewhere within the range of doses examined (Fig. 3C). NMDRCs are often U-shaped (with maximal responses of the measured endpoint observed at low and high doses) or inverted U-shaped (with maximal responses observed at intermediate doses) (Fig. 3C, *top panels*). Some cases are more complicated, with multiple points along the curve at which the slope of the curve reverses sign (Fig. 3C, *bottom left*). Nonmonotonicity is not synomymous with low dose, because there are low-dose effects that follow monotonic dose-response curves. Thus, it is not required that a study include doses that span from the true low-dose range to the high toxicological range to detect nonmonotonicity. The consequence of NMDRCs for toxicity testing is that a safe dose determined from high doses does not guarantee safety at lower, untested doses that may be closer to current human exposures.

Examples of NMDRCs from the cell culture, animal, and epidemiological literature will be discussed in detail in *Section III.C*. Importantly, our review of the literature finds that NMDRCs are common in the endocrine and EDC literature. In fact, it is plausible that, considering the mechanisms discussed below, NMDRCs are not the exception but should be expected and perhaps even common.

### A. Why is nonmonotonicity important?

NMDRCs in toxicology and in the regulatory process for EDCs are considered controversial. In addition to discussions of whether NMDRCs exist, there is also discussion of whether those that do exist have relevance to

**Figure 3.**

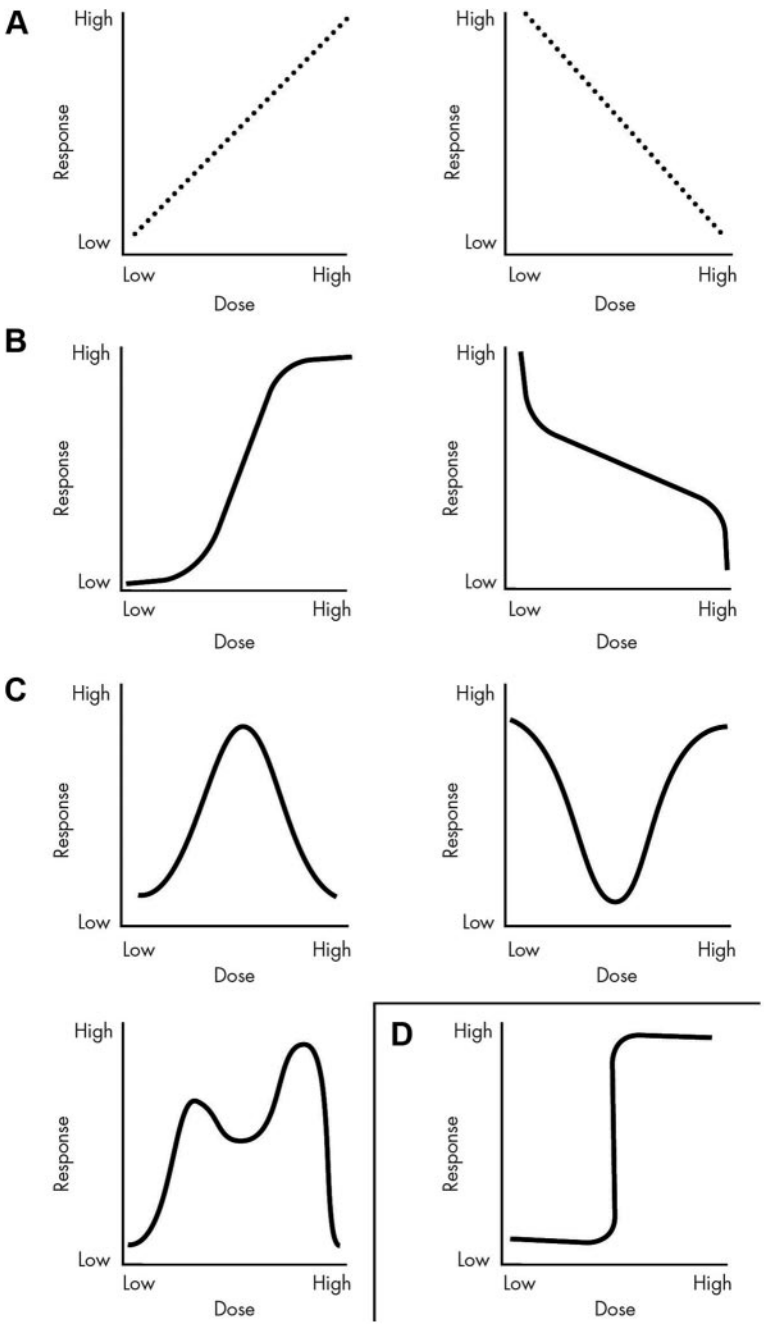



Figure 3. Examples of dose-response curves. A, Linear responses, whether there are positive or inverse associations between dose and effect, allow for extrapolations from one dose to another. Therefore, knowing the effects of a high dose permits accurate predictions of the effects at low doses. B, Examples of monotonic, nonlinear responses. In these examples, the slope of the curve never changes sign, but it does change in value. Thus, knowing what happens at very high or very low doses is not helpful to predict the effect of exposures at moderate doses. These types of responses often have a linear component within them, and predictions can be made within the linear range, as with other linear responses. C, Displayed are three different types of NMDRCs including an inverted U-shaped curve, a U-shaped curve, and a multiphasic curve. All of these are considered NMDRCs because the slope of the curve changes sign one or more times. It is clear from these curves that knowing the effect of a dose, or multiple doses, does not allow for assumptions to be made about the effects of other doses. D, A binary response is shown, where one range of doses has no effect, and then a threshold is met, and all higher doses have the same effect.

toxicological determination of putative safe exposures. In the standard practice of regulatory toxicology, the calculated safe dose, also called a reference dose, is rarely tested. In a system that is responding nonmonotonically, it is not appropriate to use a high-dose test to predict low-dose effects. Unfortunately, all regulatory testing for the effects of chemical exposures assume that this is possible. All current exposure standards employed by government agencies around the world, including the FDA and EPA, have been developed using an assumption of monotonicity (492, 493). The low-dose range, which presumably is what the general public normally experiences, is rarely, if ever, tested directly.

The standard procedure for regulatory testing typically involves a series of tests to establish the lowest dose at which an effect is observable (the LOAEL), then a dose beneath that at which no effect is observable (the NOAEL). Then a series of calculations are used to acknowledge uncertainty in the data, species differences, age differences, *etc.*, and those calculations, beginning with the LOAEL or the NOAEL, produce a reference dose that is presumed to be a safe exposure for humans (Fig. 4). Typically, the reference dose is 3- to 1000-fold lower than the NOAEL. That reference dose then becomes the allowable exposure and is deemed safe, even when it is never examined directly. For chemicals with monotonic linear dose-response curves (Fig. 3A), this may be appropriate. But for any chemicals that display nonmonotonic patterns, it is likely to lead to false negatives, *i.e.* concluding that exposure to the reference dose is safe when in fact it is not.

As described above, there are other nonlinear dose-response curves that are monotonic (Fig. 3B). These curves may also present problems for extrapolating from high doses to low doses because there is no linear relationship that can be used to predict the effects of low doses. Equally troubling for regulatory purposes are responses that have a binary response rather than a classical dose-response curve (Fig. 3D). In these types of responses, one range of doses has no effect on an endpoint, and then a threshold is met, and all higher doses have the same effect. An example is seen in the atrazine literature, where doses below 1 ppb had no effect on the size of the male larynx but doses

at or above 1 ppb produced a significant decrease in size of approximately 10–15% (336). Even doses of 200 ppb, the toxicological NOEL, produce the same effect. Thus, this all-or-none effect is observed because atrazine does not shrink the larynx; instead, it removes the stimulatory agent (*i.e.* androgens). In the absence of some threshold dose of androgen, the larynx simply remains at the unstimulated (female) size. The EPA's assessment of this study and others was that the lack of a dose-dependent response negates the importance of this effect (352). The lack of a dose response for a threshold effect like larynx size does not mean that the effects are not dose dependent; thus, understanding these types of effects and their implications for risk assessments is essential for determining the safe levels of chemicals.

It is important to mention here that the appropriateness of determining NOAEL concentrations, and therefore calculating reference doses, from exposures to endogenous hormones or EDCs has been challenged by several studies (Fig. 4A) (494–496). These studies show that hormonally active agents may still induce significant biological effects even at extremely low concentrations and that presently available analytical methods or technologies might be unable to detect relatively small magnitudes of effects. Previous discussions of this topic have shown that as the dose gets lower (and approaches zero) and the effect size decreases, the number of animals needed to achieve the power to detect a significant effect would have to increase substantially (497). Even more importantly, the assumption of a threshold does not take into account situations where an endogenous hormone is already above the dose that causes detectable effects and that an exogenous chemical (whether an agonist or antagonist) will modulate the effect of the endogenous hormone at any dose above zero (Fig. 4B). There can thus be no threshold or safe dose for an exogenous chemical in this situation. Forced identification of NOAEL or threshold doses based on the assumption that dose-response curves are always monotonic without considering the background activity of endogenous hormones and the limitations of analytical techniques supports the misconception that hormonally active agents do not have any significant biological effects at low doses. Thus, the concept that a toxic agent has a safe dose that can be readily estimated from the NOAEL derived from testing high, acutely toxic doses is overly simplistic and contradicted by data when applied to EDC (5, 497, 498).

### B. Mechanisms for NMDRCs

Previously, the lack of mechanisms to explain the appearance of NMDRCs was used as a rationale for ignoring these phenomena (492, 493). This is no longer acceptable because there are several mechanisms that have been identified and studied that demonstrate how hormones and EDCs produce nonmonotonic responses in cells, tissues, and animals. These mechanisms include cytotoxicity, cell- and tissue-specific receptors and cofactors, receptor selectivity, receptor down-regulation and desensitization, receptor competition, and endocrine negative FEEDBACK loops. These mechanisms are well understood, and by providing detailed biological insights at the molecular level into the etiology of NMDRCs, they strongly negate the presumption that has been central to regulatory toxicology that dose-response curves are by default monotonic.

#### *1. Cytotoxicity*

The simplest mechanism for NMDRCs derives from the observation that hormones can be acutely toxic at high doses yet alter biological endpoints at low, physiologically relevant doses. Experiments working at concentrations that are cytotoxic are incapable of detecting responses that are mediated by ligand-binding interactions. For example, the MCF7 breast cancer cell line proliferates in response to estradiol in the low-dose range ($10^{-12}$ to $10^{-11}$ M) and in the pharmacological and toxicological range ($10^{-11}$ to $10^{-6}$ M), but toxic responses are observed at higher doses (38). Thus, when total cell number is graphed, it displays an inverted U-shaped response to estrogen. But cells that do not contain ER, and therefore cannot be affected by the hormonal action of estradiol, also display cytotoxic responses when treated with high doses of hormone. These results clearly indicate that the effects of estradiol at high doses are toxic via non-ER-mediated mechanisms.

#### *2. Cell- and tissue-specific receptors and cofactors*

Some NMDRCs are generated by the combination of two or more monotonic responses that overlap, affecting a common endpoint in opposite ways via different pathways. For example, *in vitro* cultured prostate cell lines demonstrate a nonmonotonic response to increasing doses of androgen where low doses increase cell number and higher doses decrease cell number, thus producing an inverted U-shaped curve (499, 500). Although the parental cell expressed an inverted U-shaped dose-response curve, after a long period of inhibition, the effects on cell number could be segregated by selecting two populations of cells: one that proliferated in the absence of androgens and other cells that proliferated in the presence of high androgen levels (501). Thus, the observed inverted U-shaped response is due to actions via two independent pathways that can be separated from each other in an experimental setting (502). Similarly, estrogens have been shown to induce cell proliferation and inhibit apoptosis in several cell populations, but inhibit proliferation and induce apopto-

**Figure 4.**

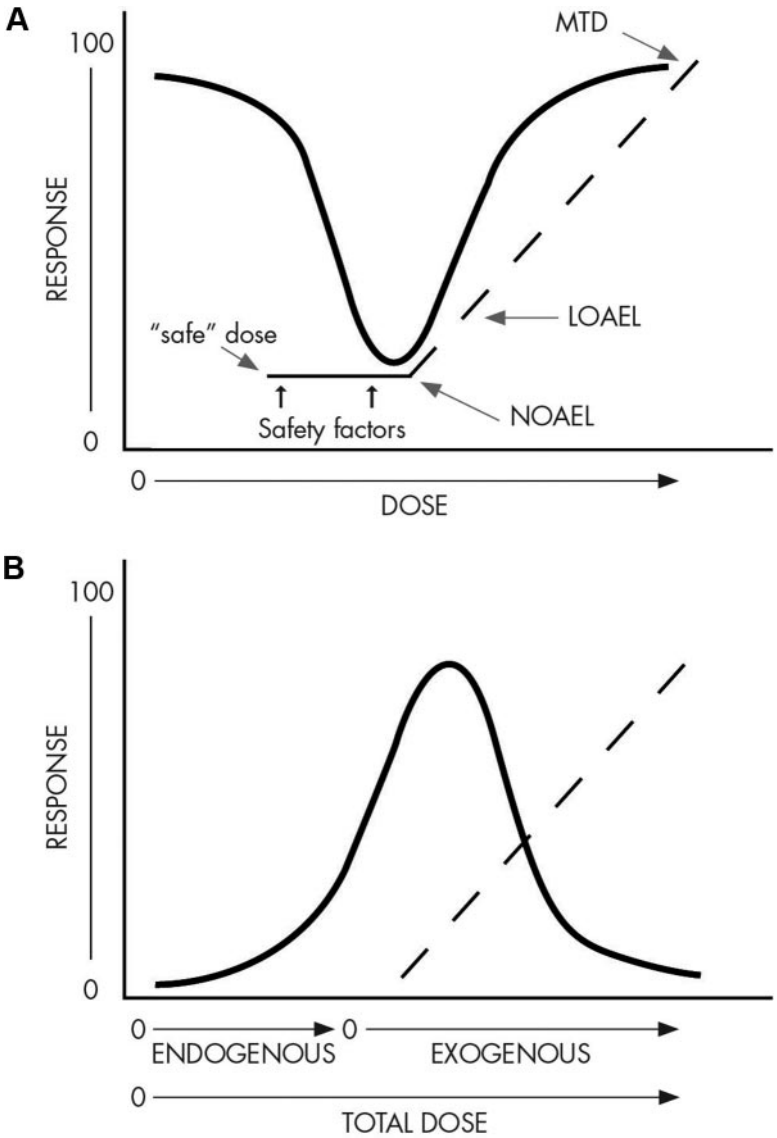



Figure 4. NOAEL, LOAEL, and calculation of a safe reference dose. A, In traditional toxicology testing, high doses are tested to obtain the maximum tolerated dose (MTD), the LOAEL, and the NOAEL. Several safety factors are then applied to derive the reference dose, *i.e.* the dose at which exposures are presumed safe. This reference dose is rarely tested directly. Yet when chemicals or hormones produce NMDRCs, adverse effects may be observed at or below the reference dose. Here, the doses that would be tested are shown by a *dotted line*, and the calculated safe dose is indicated by a *thick solid line*. The actual response, an inverted U-shaped NMDRC, is shown by a *thin solid line*. B, Experimental data indicate that EDCs and hormones do not have NOAELs or threshold doses, and therefore no dose can ever be considered safe. This is because an exogenous hormone (or EDC) could have a linear response in the tested range (*dotted line*), but because endogenous hormones are present (*thin solid line*), the effects of the exogenous hormone are always observed in the context of a hormone-containing system.

sis in others (503, 504), with the combined effect being an inverted U-shaped curve for cell number (505).

Why does one single cell type have different responses to different doses of the same hormone? The case of the prostate cell line described above is reminiscent of the results described from the transcriptome of MCF7 cells, whereby a discrete global response like cell proliferation manifests at significantly lower estrogen doses than the induction of a single marker gene (135). That a response like cell proliferation requires a significantly lower dose of hormone than the dose needed to induce a given target gene is counterintuitive but factual; it may be interpreted as consistent with the notion that metazoan cells, like cells in unicellular organisms, are intrinsically poised to divide (503, 506, 507) and that quiescence is an induced state (508, 509). The biochemical details underlying these different responses are largely unknown; however, recent studies showed that steroid receptors control only a portion of their target genes directly via promoter binding. The majority of the changes are indirect, through chromatin rearrangements (510, 511).

Why do different cell types (*in vitro* and *in vivo*) have different responses to the same hormone? One answer is that they may express different receptors, and these receptors have different responses to the same hormone. For example, some tissues express only one of the two major ER (ER$\alpha$ and ER$\beta$), and actions via these receptors are important not just for responsiveness to hormone but also for cellular differentiation and cross talk between tissue compartments (512). Yet other tissues express both ER$\alpha$ and ER$\beta$, and the effects of signaling via these two receptors often oppose each other; *i.e.* estrogen action via ER$\alpha$ induces proliferation in the uterus, but ER$\beta$ induces apoptosis (154). Complicating the situation further, different responses to a hormone can also be obtained due to the presence of different cofactors in different cell and tissue types (513, 514); these coregulators influence which genes are transcriptionally activated or repressed in response to the presence of hormone. They can also influence ligand selectivity of the receptor and DNA-binding capacity, having tremendous impact on the ability of a hormone to have effects in different cell types (105, 515, 516).

Although much of these activities occur on a biochemical level, *i.e.* at the receptor, there is also evidence that nonmonotonicity can originate at the level of tissue organization. The mammary gland has been used as a model to study inter- and intracompartmental effects of hormone treatment: within the ductal epithelium, estro-

gen has distinct effects during puberty, both inducing proliferation, which causes growth of the ductal tree, and inducing apoptosis, which is required for lumen formation (517, 518); in cell culture, the presence of stromal cells can also enhance the effects of estrogen on epithelial cells (519, 520), suggesting that stromal-epithelial compartmental interactions can mediate the effects of estrogen.

#### *3. Receptor selectivity*

NMDRCs can occur because of differences in receptor affinity, and thus the selectivity of the response, at low *vs.* high doses. For example, at low doses, BPA almost exclusively binds to the ER (including mER), but at high doses it can also bind weakly to other hormone receptors, like androgen receptor and thyroid hormone receptor (249, 521). This type of receptor nonselectivity is quite common for EDCs, and it has been proposed that binding to different receptors may be an explanation for the diverse patterns of disease observed after EDC exposures (522). In fact, several of the chemicals shown to have low-dose effects are known to act via multiple receptors and pathways (Table 3). Thus, the effects seen at high doses can be due to action via the binding of multiple receptors, compared with the effects of low doses, which may be caused by action via only a single receptor or receptor family.

#### *4. Receptor down-regulation and desensitization*

When hormones bind to nuclear receptors, the ultimate outcome is a change in the transcription of target genes. When the receptor is bound by ligand, an increase in response is observed; as discussed previously in this review, the relationship between hormone concentration and the number of bound receptors, as well as the relationship between the number of bound receptors and the biological effect, is nonlinear (38). After the nuclear receptor is bound by hormone and transcription of target genes has occurred (either due to binding of the receptor at a DNA response element or the relief of a repressive event on the DNA), the reaction eventually must cease; *i.e.* the bound receptor must eventually be inactivated in some way. Thus, nuclear hormone receptors are ubiquitinated and degraded, usually via the proteasome (523). Importantly, the role of the hormone in receptor degradation differs depending on the hormone; binding of estrogen, progesterone, and glucocorticoid mediates the degradation of their receptors (524–526), whereas the presence of hormone may actually stabilize some receptors and prevent degradation (527), and other receptors are degraded without ligand (528). As hormone levels rise, the number of receptors being inactivated and degraded also rises, and eventually the number of receptors being produced cannot maintain the pace of this degradation pathway (523). Furthermore, the internalization and degradation of receptors can also influence receptor production, leading to an even stronger down-regulation of receptor (529). In the animal, the role of receptor down-regulation is actually quite complex, because signaling from one hormone receptor can influence protein levels of another receptor; *i.e.* ER signaling can promote degradation of the glucocorticoid receptor by increasing the expression of enzymes in the proteasome pathway that degrade it (530).

There is also the issue of receptor desensitization, a process whereby a decrease in response to a hormone is not due to a decrease in the number of available receptors but instead due to the biochemical inactivation of a receptor (531). Desensitization typically occurs when repeated or continuous exposure to ligand occurs. Normally seen with membrane-bound G protein-coupled receptors, the activation of a receptor due to ligand binding is quickly followed by the uncoupling of the activated receptor from its G proteins due to phosphorylation of these binding partners (532). Receptor desensitization has been observed for a range of hormones including glucagon, FSH, human chorionic gonadotropin, and prostaglandins (533). Importantly, desensitization and down-regulation can occur in the same cells for the same receptor (534), and therefore, both can play a role in the production of NMDRCs.

#### *5. Receptor competition*

Mathematical modeling studies suggest that the mixture of endogenous hormones and EDCs establishes a natural environment to foster NMDRCs. Using mathematical models, Kohn and Melnick (42) proposed that when EDC exposures occur in the presence of endogenous hormone and unoccupied hormone receptors, some unoccupied receptors become bound with the EDC, leading to an increase in biological response (*i.e.* increased expression of a responsive gene, increased weight of an organ, *etc.*). At low concentrations, both the endogenous hormone and the EDC bind to receptors and activate this response, but at high doses, the EDC can outcompete the natural ligand. The model predicts that inverted U-shaped curves would occur regardless of the binding affinity of the EDC for the receptor and would be abolished only if the concentration of natural hormone were raised such that all receptors were bound.

#### *6. Endocrine negative FEEDBACK loops*

In several cases, the control of hormone synthesis is regulated by a series of positive- and negative feedback loops. Several hormones are known to control or influence their own secretion using these feedback systems. In one example, levels of insulin are known to regulate glucose uptake by cells. Blood glucose levels stimulate insulin pro-

duction, and as insulin removes glucose from circulation, insulin levels decline. Thus, NMDRCs can occur as the free/available ligand and receptor concentrations are influenced by one another. In another example, thyroid hormone secretion is stimulated by TSH, and thyroid hormone suppresses TSH; thus, feedback between these two hormones allows thyroid hormone to be maintained in a narrow dose range.

Several studies indicate that these negative feedback loops could produce NMDRCs when the duration of hormone administration is changed (535). For example, short exposures of estrogen induce proliferation in the uterus and pituitary, but longer hormone regimens inhibit cell proliferation (236, 536). Thus, the outcome is one where exposure to a single hormone concentration stimulates an endpoint until negative feedback loops are induced and stimulation ends (537).

#### *7. Other downstream mechanisms*

Removing the variability that can come from examining different cell types, or even single cell types in the context of a tissue, studies of cultured cells indicate that different gene profiles are affected by low doses of hormone compared with higher doses. In a study of the genes affected by low *vs.* higher doses of estrogen, researchers found that there were a small number of genes in MCF7 breast cancer cells with very high sensitivity to low doses of estradiol (10 pM) compared with the total number of genes that were affected by higher (30 or 100 pM) exposures (538). But the surprising finding was the pattern of estradiol-induced *vs.* estradiol-suppressed gene expression at high and low doses; when 10 pM was administered, the number of estradiol-suppressible genes was approximately three times higher than the number of estradiol-inducible genes. However, the overall profile of the number of estradiol-suppressible genes was approximately half the total number of estradiol-inducible genes. This observation suggests that low doses of estrogen selectively target a small subset of the total number of estrogen-sensitive genes and that the genes affected by low doses are most likely to be suppressed by that treatment. The mechanisms describing how low doses of estrogen differently affect the expression of genes compared with higher doses have yet to be elucidated, but low doses of estradiol inhibit expression of apoptotic genes (539), indicating that which genes are affected by hormone exposure is relevant to understand how low doses influence cellular activities.

### C. Examples of nonmonotonicity

#### *1. Examples of NMDRCs from cell culture*

A tremendous amount of theoretical and mathematical modeling has been conducted to understand the production of nonlinear and nonmonotonic responses (42, 540). These studies and others suggest that the total number of theoretical response curves is infinite. Yet this does not mean that the occurrence of NMDRCs is speculative; these types of responses are reported for a wide variety of chemicals. Cell culture experiments alone provide hundreds of examples of nonmonotonic responses (see Table 6 for examples). In the natural hormone category, many different hormones produce NMDRCs; this is clearly not a phenomenon that is solely attributable to estrogen and androgen, the hormones that have been afforded the most attention in the dose-response literature. Instead, NMDRCs are observed after cells are treated with a range of hormones, suggesting that this is a fundamental and general feature of hormones.

Chemicals from a large number of categories with variable effects on the endocrine system also produce NMDRCs in cultured cells. These chemicals range from components of plastics to pesticides to industrial chemicals and even heavy metals. The mechanisms for nonmonotonicity discussed in *Section III.B* are likely explanations for the NMDRCs reported in a range of cell types after exposure to hormones and EDCs. Table 6 provides only a small number of examples from the literature, and it should be noted that because these are studies of cells in culture, most of these studies typically examined only a few types of outcomes: cell number (which could capture the effects of a chemical on cell proliferation, apoptosis, or both), stimulation or release of another hormone, and regulation of target protein function, often examined by measuring the phosphorylation status of a target.

#### *2. Examples of NMDRCs in animal studies*

Some scientists suggest that nonmonotonicity is an artifact of cell culture, however, a large number of NMDRCs have been observed in animals after administration of natural hormones and EDCs, refuting the hypothesis that this is a cell-based phenomenon only. Similar to what has been observed in cultured cells, the NMDRCs observed in animals also span a large range of chemicals, model organisms, and affected endpoints (Table 7). These results underscore the biological importance of the mechanisms of nonmonotonicity that have been largely worked out *in vitro*.

Although NMDRCs attributable to estrogen treatment are well documented, the induction of NMDRCs is again observed to be a general feature of hormone treatment; a wide range of hormones produce these types of responses in exposed animals. Importantly, a number of pharmaceutical compounds with hormone-mimicking or endocrine-disrupting activities also produce NMDRCs. Finally, as expected from the results of cell culture

**TABLE 6.** Examples of NMDRCs in cell culture experiments

| Chemicals by chemical class | Nonmonotonic effect | Cell type | Refs. |
|---|---|---|---|
| Natural hormones | | | |
| 17β-Estradiol | Cell number | MCF7 breast cancer cells | 135, 716 |
| | Dopamine uptake | Fetal hypothalamic cells (primary) | 717 |
| | pERK levels, prolactin release | GH3/B6/F10 pituitary cells | 41, 718, 719 |
| | β-Hexosaminidase release | HMC-1 mast cells | 720 |
| | Cell number | Vascular smooth muscle cells | 721 |
| | Production of L-PGDS, a sleep-promoting substance | U251 glioma cells | 722 |
| 5α-Dihydrotestosterone | Cell number | LNCaP-FGC prostate cancer cells | 499 |
| | Cell number, kinase activity | Vascular smooth muscle cells | 721 |
| 5α-Androstenedione | Cell number | LNCaP-FGC prostate cancer cells | 499 |
| Corticosterone | Mitochrondrial oxidation, calcium flux | Cortical neurons (primary) | 723 |
| Insulin | Markers of apoptosis (in absence of glucose) | Pancreatic β-cells (primary) | 724 |
| Progesterone | Cell number | LNCaP-FGC prostate cancer cells | 499 |
| Prolactin | Testosterone release | Adult rat testicular cells (primary) | 725 |
| hCG | Testosterone release | Adult rat testicular cells (primary) | 725 |
| $T_3$ | Rate of protein phosphorylation | Cerebral cortex cells (primary, synaptosomes) | 726 |
| | *LPL* mRNA expression | White adipocytes (rat primary) | 727 |
| GH | *IGF-I* expression | Hepatocytes (primary cultures from silver sea bream) | 728 |
| Pharmaceutical hormones | | | |
| DES | Cell number | MCF7 breast cancer cells | 716 |
| | Prolactin release | GH3/B6/F10 pituitary cells | 41 |
| Ethinyl estradiol | CXCL12 secretion | MCF7 breast cancer cells, T47D breast cancer cells | 729 |
| R1881 (synthetic androgen) | Cell number | LNCaP-FGC cells | 499 |
| Trenbolone | Induction of micronuclei | RTL-W1 fish liver cells | 730 |
| Plastics | | | |
| BPA | Cell number | MCF7 breast cancer cells | 135, 716 |
| | Dopamine efflux | PC12 rat tumor cells | 40 |
| | pERK levels, intracellular $Ca^{2+}$ changes, prolactin release | GH3/B6/F10 pituitary cells | 41, 718 |
| | Cell number | LNCaP prostate cancer cells | 731 |
| DEHP | Number of colonies | *Escherichia coli* and *B. subtilis* bacteria | 732 |
| Di-*n*-octyl phthalate | Number of colonies | *E. coli* and *B. subtilis* bacteria | 732 |
| Detergents, surfactants | | | |
| Octylphenol | Cell number | MCF7 breast cancer cells | 716 |
| | Dopamine uptake | Fetal hypothalamic cells (primary) | 717 |
| | pERK levels | GH3/B6/F10 pituitary cells | 718 |
| | hCG-stimulated testosterone levels | Leydig cells (primary) | 733 |
| Propylphenol | pERK levels | GH3/B6/F10 pituitary cells | 718 |
| Nonylphenol | pERK levels, prolactin release | GH3/B6/F10 pituitary cells | 41, 718 |
| | β-Hexosaminidase release | HMC-1 mast cells | 720 |
| | Cell number | MCF7 breast cancer cells | 135 |
| PAH | | | |
| Phenanthrene | All-trans retinoic acid activity | P19 embryonic carcinoma cells | 734, 735 |
| Benz(a)acridine | All-trans retinoic acid activity | P19 embryonic carcinoma cells | 734 |
| Naphthalene | hCG-stimulated testosterone | Pieces of goldfish testes | 736 |
| B-naphthoflavone | hCG-stimulated testosterone | Pieces of goldfish testes | 736 |
| Retene | hCG-stimulated testosterone | Pieces of goldfish testes | 736 |
| Heavy metals | | | |
| Lead | Estrogen, testosterone, and cortisol levels | Postvitellogenic follicles (isolated from catfish) | 737 |
| Cadmium | Expression of angiogenesis genes | Human endometrial endothelial cells | 738 |

(*Continued*)

**TABLE 6.** Continued

| Chemicals by chemical class | Nonmonotonic effect | Cell type | Refs. |
|---|---|---|---|
| Phytoestrogens and natural antioxidants | | | |
| Genistein | Cell number | Caco-2BBe colon adenocarcinoma cells | 739 |
| | CXCL12 secretion, cell number | T47D breast cancer cells | 729 |
| | Cell number, cell invasion, MMP-9 activity | PC3 prostate cancer cells | 740 |
| | pJNK levels, $Ca^{2+}$ flux | GH3/B6/F10 pituitary cells | 719 |
| Coumesterol | Prolactin release, pERK levels | GH3/B6/F10 pituitary cells | 719 |
| Daidezin | Prolactin release, pERK levels | GH3/B6/F10 pituitary cells | 719 |
| | Cell number | MCF7 breast cancer cells | 135 |
| | Cell number | LoVo colon cancer cells | 741 |
| Resveratrol | Expression of angiogenesis genes | Human umbilical vein endothelial cells | 742 |
| Trans-resveratrol | pERK levels, $Ca^{2+}$ flux | GH3/B6/F10 pituitary cells | 719 |
| Artelastochromene | Cell number | MCF7 breast cancer cells | 743 |
| Carpelastofuran | Cell number | MCF7 breast cancer cells | 743 |
| Biochanin A | Induction of estrogen-sensitive genes in the presence of testosterone | MCF7 breast cancer cells | 744 |
| Licoflavone C | Induction of estrogen-sensitive genes | Yeast bioassay | 745 |
| Quercetin | Aromatase activity | H295R adrenocortical carcinoma cells | 746 |
| | Cell number | SCC-25 oral squamous carcinoma cells | 747 |
| Dioxin | | | |
| TCDD | Cell number, gene expression | M13SV1 breast cells | 748 |
| PCB | | | |
| PCB-74 | Cell viability, GnRH peptide levels | GT1-7 hypothalamic cells | 749 |
| PCB-118 | Cell viability, GnRH peptide levels | GT1-7 hypothalamic cells | 749 |
| Aroclor 1242 (PCB mixture) | $\beta$-Hexosaminidase release | HMC-1 mast cells | 720 |
| POP mixture | Apoptosis of cumulus cells | Oocyte-cumulus complexes (primary, isolated from pigs) | 750 |
| Herbicides | | | |
| Glyphosphate-based herbicide (Round-Up) | Cell death, aromatase activity, ER$\beta$ activity | HepG2 liver cells | 751 |
| Atrazine | Cell number | IEC-6 intestinal cells | 752 |
| Insecticides | | | |
| Endosulfan | Cell number | IEC-6 intestinal cells | 752 |
| | $\beta$-Hexosaminidase release | HMC-1 mast cells | 720 |
| | ATPase activity of P-glycoprotein | CHO cell extracts | 753 |
| Diazinon | Cell number | IEC-6 intestinal cells | 752 |
| Dieldrin | $\beta$-Hexosaminidase release | HMC-1 mast cells | 720 |
| DDT | Cell number | MCF7 breast cancer cells | 144 |
| DDE | $\beta$-Hexosaminidase release | HMC-1 mast cells | 720 |
| | Prolactin release | GH3/B6/F10 pituitary cells | 41 |
| 3-Methylsulfonyl-DDE | Cortisol and aldosterone release, expression of steroidogenic genes | H295R adrenocortical carcinoma cells | 754 |
| Fungicides | | | |
| Hexachlorobenzene | Transcriptional activity in the presence of DHT | PC3 prostate cancer cells | 755 |
| Prochloraz | Aldosterone, progesterone, and corticosterone levels; expression of steroidogenic genes | H295R adrenocortical cells | 756 |
| Ketoconazole | Aldosterone secretion | H295R adrenocortical cells | 757 |
| Fungicide mixtures | Aldosterone secretion | H295R adrenocortical cells | 757 |
| PBDE | | | |
| PBDE-49 | Activation of ryanodine receptor 1 | HEK293 cell (membranes) | 758 |
| PBDE-99 | Expression of *GAP43* | Cerebral cortex cells (primary) | 759 |

Due to space concerns, we have not elaborated on the shape of the curve (U, inverted U, or other nonmonotonic shape) or the magnitude of observed effects in this table. CXCL12, Chemokine (C-X-C motif) ligand 12; DEHP, bis(2-ethylhexyl) phthalate; DHT, dihydrotestosterone; hCG, human chorionic gonadotropin; MMP, matrix metalloproteinase; PAH, polyaromatic hydrocarbons; PBDE, polybrominated diphenyl ethers; PCB, polychlorinated biphenyl; pERK, phospho-ERK; PGDS, prostaglandin-D synthase; pJNK, phospho-c-Jun N-terminal kinase.

**TABLE 7.** Examples of NMDRCs in animal studies

| Chemicals by chemical class | Nonmonotonic effect | Organ/sex/animal | Refs. |
|---|---|---|---|
| Natural hormones | | | |
| 17$\beta$-Estradiol | Morphological parameters | Mammary gland/female/mice | 138, 541 |
| | Accumulation of cAMP | Pineal/female/rats | 760 |
| | Prostate weight | male/mice | 689 |
| | Uterine weight | female/mice | 761 |
| | Antidepressant effects, measured by immobility assay | Behavior/male/mice | 762 |
| | Nocturnal activity, gene expression in preoptic area | Brain and behavior/female/mice | 763 |
| Corticosterone | Spatial memory errors | Behavior/male/rats | 764 |
| | Cholinergic fiber loss in cortex after treatment with neurodegenerative drugs | Brain/male/rats | 765 |
| | Mitochondrial metabolism | Muscle/male/rats: strain differences | 766 |
| | Contextual fear conditioning | Behavior/male/rats | 767 |
| | Locomotor activity | Behavior/male/captive Adelie penguins | 768 |
| Glucocorticoid | $Na^+/K^+$-ATPase activity | Brain/tilapia (fish) | 769 |
| Testosterone | $Na^+/K^+$-ATPase activity | Brain/tilapia (fish) | 769 |
| | Gonadotropin subunit gene expression | Pituitary/sexually immature goldfish | 770 |
| 11$\beta$-Hydroxyandrosterone | Gonadotropin subunit gene expression | Pituitary/sexually immature goldfish | 770 |
| $T_4$ | Bone growth | Tibia/male/rats with induced hypothyroidism | 771 |
| Leptin | Insulin production (in the presence of glucose) | Pancreas/male/rats | 560 |
| Oxytocin | Infarct size, plasma LDH levels, creatine kinase activity after ischemia/ reperfusion injury | Brain and blood/male/rats | 772 |
| | Memory retention | Behavior/male/mice | 773 |
| Melatonin | Brain infarction and surviving neuron number after injury | Brain/female/rats | 774 |
| Dopamine | Memory | Brain/both/rhesus monkey | 775 |
| | Neuronal firing rate | Brain/male/rhesus monkey | 776 |
| Pharmaceutical | | | |
| DES | Sex ratio, neonatal body weight, other neonatal development | Mice | 777 |
| | Adult prostate weight | Male/mice | 689 |
| | Uterine weight | Female/mice | 761 |
| | Expression of PDGF receptor | Testes/male/rats | 778 |
| | Morphological parameters | Mammary gland/male and female/ mice | 779 |
| Estradiol benzoate | Dorsal prostate weight, body weight | Male/rats | 780 |
| | Sexual behaviors, testes morphology | Male/zebra finches (birds) | 781 |
| Ethinyl estradiol | GnRH neurons | Brain/zebrafish | 782 |
| Tamoxifen | Uterine weight | Female/mice | 761 |
| Fluoxetine (antidepressant) | Embryo number | *Potamopyrgus antipodarum* (snails) | 783 |
| Fadrozole (aromatase inhibitor) | Aromatase activity | Ovary/female/fathead minnows | 784 |
| Plastics | | | |
| BPA | Fertility | Reproductive axis /female/mice | 316 |
| | Reproductive behaviors | Behavior/male/rats | 785 |
| | Protein expression | Hepatopancreas/male/*Porcellio scaber* (isopod) | 786 |
| | Timing of vaginal opening, tissue organization of uterus | Reproductive axis/female/mice | 577 |
| | Expression of receptors in embryos | Brain and gonad/both/ mice | 787 |
| DEHP | Aromatase activity | Hypothalamus/male/rats | 788 |
| | Cholesterol levels | Serum/male/rats | 569 |
| | Timing of puberty | Reproductive axis /male/rats | 789 |
| | Body weight at birth, vaginal opening, and first estrous | Female/rats | 790 |
| | Seminal vesicle weight, epididymal weight, testicular expression of steroidogenesis genes | Male/rats | 791 |
| | Responses to allergens, chemokine expression | Skin/male/mice | 792 |

(*Continued*)

**TABLE 7.** Continued

| Chemicals by chemical class | Nonmonotonic effect | Organ/sex/animal | Refs. |
|---|---|---|---|
| Detergents, surfactants | | | |
| Nonylphenol ethoxylate | Fecundity | *Biomphalaria tenagophila* (snails) | 793 |
| Octylphenol | Embryo production | *P. antipodarum* (snails) | 794 |
| | Spawning mass and egg numbers | *Marisa cornuarietis* (snails) | 795 |
| Semicarbazide | Timing of preputial separation, serum DHT | Male/rats | 796 |
| Antimicrobial | | | |
| Triclocarban | Fecundity | *P. antipodarum* (snails) | 797 |
| PCB | | | |
| Mixture of PCB | Corticosterone levels | Male/kestrels (birds) | 798 |
| Environmental PCB mixture | Corticosterone levels | Female/tree swallows (birds) | 799 |
| UV filters | | | |
| Octyl methoxycinnamate | Activity, memory | Behavior/both/rats | 800 |
| Aromatic hydrocarbons | | | |
| B-naphthoflavone | Testosterone | Plasma/male/goldfish | 736 |
| Toluene | Locomotor activity | Behavior/male/rats | 801 |
| Dioxins | | | |
| TCDD | Cell-mediated immunity | Immune system/male/ rats | 802 |
| | Proliferation after treatment with chemical carcinogen | Liver/female/rats | 803 |
| Heavy metals | | | |
| Cadmium | Expression of metallothionein, *pS2/TFF1* | Intestine and kidney/ female/rats | 804 |
| | Activity of antioxidant enzymes | Earthworms | 805 |
| | Size parameters, metamorphic parameters | *Xenopus laevis* | 806 |
| Lead | Growth, gene expression | *Vicia faba* seedlings (plant) | 807 |
| | Retinal neurogenesis | Eye and brain/female/rats | 808 |
| Selenium | DNA damage, apoptotic index | Prostate/male/dogs | 809 |
| | Hatching failure | Eggs/red-winged blackbirds (wild population) | 810 |
| Phytoestrogens | | | |
| Genistein | Aggressive, defensive behaviors | Behavior/male/mice | 811 |
| | Retention of cancellous bone after ovariectomy | Tibia bones/female/rat | 812 |
| | Expression of *OPN*, activation of Akt | Prostate/male/mice | 740 |
| Resveratrol | Angiogenesis | Chorioallantoic membrane/chicken embryos | 742 |
| | Ulcer index after chemical treatment, expression of gastroprotective genes | Stomach/male/mice | 813 |
| Phytochemicals | | | |
| Phlorizin | Memory retention | Behavior/male/mice | 814 |
| Herbicides | | | |
| Atrazine | Time to metamorphosis | Thyroid axis/*Rhinella arenarum* (South American toad) | 815 |
| | Survivorship patterns | Four species of frogs | 363 |
| | Growth parameters | *Bufo americanus* | 816 |
| Pendimethalin | Expression of *AR*, *IGF-I* | Uterus/female/mice | 817 |
| Commercial mixture with mecoprop, 2,4-dichlorophenoxyacetic acid and dicamba | Number of implantation sites, number of live births | Female/mice | 818 |
| Simazine | Estrous cyclicity | Reproductive axis/female/rat | 819 |
| Insecticides | | | |
| Permethrin | Dopamine transport | Brain/male/mice | 820 |
| Heptachlor | Dopamine transport | Brain/male/mice | 820 |
| DDT | Number of pups, sex ratios, neonatal body weight, male anogenital distance | Mice | 777 |
| Methoxychlor | Number of pups, anogenital distance (males and females), neurobehaviors (males and females) | Mice | 777 |
| Chlorpyrifos | Body weight | Male/rats | 821 |
| | Antioxidant enzyme activity | *Oxya chinensis* (locusts) | 822 |
| Malathion | Antioxidant enzyme activity | *O. chinensis* (locusts) | 822 |

(*Continued*)

**TABLE 7.** Continued

| Chemicals by chemical class | Nonmonotonic effect | Organ/sex/animal | Refs. |
|---|---|---|---|
| Fungicides | | | |
| Carbendazim | Liver enzymes, hematology parameters | Blood and liver/male/rats | 823 |
| Chlorothalonil | Survival, immune response, corticosterone levels | Several amphibian species | 686 |
| Vinclozolin | Protein expression | Testes/male/*P. scaber* (isopod) | 786 |

Due to space concerns, we have not elaborated on the shape of the curve (U, inverted U, or other nonmonotonic shape) or the magnitude of observed effects in this table. DEHP, Bis(2-ethylhexyl) phthalate; DHT, dihydrotestosterone; LDH, lactate dehydrogenase; PCB, polychlorinated biphenyl; PDGF, platelet-derived growth factor.

experiments, chemicals with many different modes of action generate NMDRCs in treated animals.

Perhaps most striking is the range of endpoints affected, from higher-order events such as the number of viable offspring (which could be due to alterations in the reproductive tissues themselves or the reproductive axis), to behavioral effects, to altered organ weights, and to lower-order events such as gene expression. The mechanisms responsible for these nonmonotonic phenomena may be similar to those studied in cell culture systems, although additional mechanisms are likely to be operating *in vivo* such as alterations in tissue organization (541) and the interactions of various players in the positive and negative feedback loops of the endocrine system.

### *3. Examples of NMDRCs in the epidemiology literature*

Perhaps not surprisingly, natural hormones produce NMDRCs in human populations as well (Table 8). Although the methods needed to detect NMDRCs in humans are specific to the field of epidemiology, these results sup-

**TABLE 8.** NMDRCs for natural hormones identified in the epidemiology literature

| Hormone | Affected endpoint | NMDRC | Study subjects | Refs. |
|---|---|---|---|---|
| Testosterone (free) | Incidence of coronary events | Incidence of 25% at extremes of exposure, 16% at moderate exposure | Rancho Bernardo Study participants, women aged 40+ (n = 639) | 824 |
| | Depression | Hypo- and hypergonadal had higher depression scores than those with intermediate free testosterone | Androx Vienna Municipality Study participants, manual workers, men aged 43–67 (n = 689) | 825 |
| PTH | Mortality | ~50% excess risk for individuals with low or high iPTH | Hemodialysis patients (n = 3946) | 826 |
| | Risk of vertebral or hip fractures | ~33% higher for low or high iPTH compared to normal levels | Elderly dialysis patients (n = 9007) | 827 |
| TSH | Incidence of Alzheimer's disease | About double the incidence in lowest and highest tertile in women (no effects observed in men) | Framingham Study participants (elderly) (n = 1864, 59% women) | 828 |
| Leptin | Mortality | Mortality ~10% higher for lowest and highest leptin levels | Framingham Heart Study participants (elderly) (n = 818, 62% women) | 563 |
| Insulin | Coronary artery calcification | Higher for low and high insulin area under the curve measures. | Nondiabetic patients with suspected coronary heart disease, cross-sectional (n = 582) | 829 |
| | Mortality (noncardiovascular only) | Relative risk ~1.5 for highest and lowest fasting insulin levels | Helsinki Policemen Study participants, men aged 34–64 (n = 970) | 830 |
| Cortisol | BMI, waist circumference | Low cortisol secretion per hour for individuals with highest and lowest BMI, waist circumference | Whitehall II participants, adults, cross-sectional (n = 2915 men; n = 1041 women) | 831 |
| | Major depression (by diagnostic interview) | Slight increases at extremes of cortisol | Longitudinal Aging Study Amsterdam participants, aged 65+, cross-sectional (n = 1185) | 832 |

BMI, Body mass index; iPTH, intact PTH; PTH, parathyroid hormone.

port the idea that NMDRCs are a fundamental feature of hormones. Importantly, it should be noted that most of the individuals surveyed in studies examining the effects of natural hormones have a disease status or are elderly. This of course does not mean that natural hormones induce NMDRCs in only these select populations but may instead be a reflection of the types of individuals available for these studies (for example, there are very few clinical events in younger people).

NMDRCs observed in the epidemiology literature from human populations exposed to EDCs are now starting to receive attention (Table 9). Here, most reports of NMDRCs come from studies of healthy individuals exposed to persistent organic pollutants POPs, chemicals that do not easily degrade and consequently bioaccumulate in human and animal tissues (542). These POPs do encompass a range of chemical classes including components of plastics, pesticides, and industrial pollutants. A large number of these studies have focused on endpoints that are relevant to metabolic disease, and together, these studies show that there is a recurring pattern of NMDRCs related to POPs and disease. Of course, not every study of POPs shows NMDRCs, and this is probably due to the distribution of EDCs in the populations examined.

In addition to the studies that show strong evidence for NMDRCs in human populations, there is also a subset of studies that provide suggestive evidence for nonmonotonic relationships between EDCs and human health endpoints (Table 9). In fact, the authors of many of these papers clearly identify U- or inverted U-shaped dose-response curves. However, when authors do not perform the appropriate statistical tests to verify the presence of a NMDRC, there is some ambiguity in their conclusions. The usual cross-sectional *vs.* prospective design dichotomy in epidemiology also is a factor that can influence the strength of a NMDRC, or prevent the detection of one at all. This disjunction in design is often incongruous with EDC exposure studies because we often know very little about clearance rates of the chemical, interactions with adiposity, and changes to these factors with age and gender. Yet regardless of any possible weaknesses in these studies, they provide supportive evidence that NMDRCs are observed in human populations.

Because these reports of NMDRCs in human populations are relatively new, few mechanisms have been proposed for these phenomena. Why would risk curves be nonmonotonic over the dose distribution observed in human populations? Why would individuals with the highest exposures have less severe health outcomes compared with individuals with more moderate exposures? One plausible explanation is that the same mechanisms for NMDRCs in animals and cell cultures operate in human populations: chronic exposures to high doses can activate negative feedback loops, activate receptors that promote changes in different pathways that diverge on the same endpoint with opposing effects, or produce some measure of toxicity. Accidental exposures of very large doses may not behave the same as background doses for a variety of reasons, including the toxicity of high doses; these large doses tend to occur over a short time (and therefore more faithfully replicate what is observed in animal studies after controlled administration).

Another explanation is that epidemiology studies, unlike controlled animal studies, examine truly complex mixtures of EDCs and other environmental chemicals. Some chemical exposures are likely to be correlated due to their sources and their dynamics in air, water, soil, and living organisms that are subsequently eaten. Therefore, intake of these chemicals may produce unpredicted, likely nonlinear outcomes whether the two chemicals act via similar or different pathways.

The design of observational epidemiological studies is fundamentally different from studies of cells or animals, in that the EDC exposure distributions are given, rather than set by the investigator. In particular, as shown in Fig. 5, different epidemiological populations will have different ranges of exposure, with the schematic example showing increasing risk in a population with the lowest exposures (labeled group A), an inverted U-shaped risk in a moderate dose population (labeled group B), and an inverse risk in a population with the highest exposures (labeled group C). An additional example is provided (labeled group D) in which an industrial spill shows high risk, but the comparison with the entire unaffected population with a wide variety of risk levels due to differential background exposure could lead to a high- or a low-risk reference group and a wide variety of possible findings.

It is reasonable to suggest that even though epidemiological studies are an assessment of exposures at a single time point, many of these pollutants are persistent, and therefore a single measure of their concentration in blood may be a suitable surrogate for long-term exposures. The movement of people from relatively low- to higher-exposure groups over time depend on refreshed exposures, clearance rates, and individual differences in ability to handle exposures (*i.e.* due to genetic susceptibilities, amount of adipose tissue where POPs can be stored, *etc.*).

Figure 5 therefore further illustrates that observational epidemiological studies yield the composite effect of varying mixtures of EDCs at various exposure levels for various durations, combining acute and chronic effects. These studies are important, however, in that they are the only way to study EDC effects in the long term in intact humans, as opposed to studying signaling pathways, cells,

**TABLE 9.** NMDRCs for EDCs identified in the epidemiology literature

| Chemicals by chemical class | Affected endpoint | NMDRC | Study subjects | Refs. |
|---|---|---|---|---|
| Insecticides | | | | |
| Trans-nonachlor | Diabetes incidence | Highest risk in groups with intermediate exposures (quartile 2) | CARDIA participants, case-control study (n = 90 cases and n = 90 controls) | 833 |
| | Telomere length in peripheral leukocytes | Increased length in intermediate exposures (quintile 4) | Adults aged 40+ (Korea, n = 84) | 591 |
| *p,p'*-DDE | BMI, triglyceride levels, HDL cholesterol | Highest risk in groups with intermediate exposures (quartile 3) | CARDIA participants (n = 90 controls from nested case control study) | 590 |
| | Risk of rapid infant weight gain | For infants born to women of normal weight prepregnancy, risk is highest with intermediate exposures. | Infants from Childhood and the Environment project, Spain (n = 374 from normal prepregnancy weight mothers; n = 144 from overweight mothers) | 834 |
| | Telomere length in peripheral leukocytes | Increased length with intermediate exposures (quintile 4) | Adults aged 40+ (Korea, n = 84) | 591 |
| Oxychlordane | Bone mineral density of arm bones | With low exposures, fat mass had inverse associations with bone mineral density; with high exposures, fat mass had positive associations with bone mineral density. | NHANES 1999–2004 participants, aged 50+ (n = 679 women, n = 612 men) | 835 |
| Plastics | | | | |
| Mono-methyl phthalate (MMP) | Atherosclerotic plaques | Increased risk in intermediate exposure groups (quintiles 2–4) | Adults aged 70, living in Sweden (n = 1016) | 836 |
| Perfluorinated compounds | | | | |
| PFOA | Arthritis (self-reported) | Increased risk in intermediate exposure groups (quartile 2) | NHANES participants, aged 20+ (both sexes, n = 1006) | 837 |
| Fire retardants | | | | |
| PBB-153 | Blood triglyceride levels | Increased risk in intermediate exposure groups (quartile 2) | NHANES participants, aged 12+ (n = 637) | 604 |
| PBDE-153 | Prevalence of diabetes, | Prevalence of diabetes highest in intermediate groups (quartiles 2–3 relative to individuals with undetectable levels) | NHANES participants, aged 12+ (n = 1367) | 604 |
| | Prevalence of metabolic syndrome, levels of blood triglycerides | Prevalence of metabolic syndrome highest in intermediate exposure groups (quartile 2 relative to individuals with undetectable levels); blood triglycerides highest in low exposure groups (quartile 1 relative to individuals with undetectable levels) | NHANES participants, aged 12+ (n = 637) | 604 |
| PCB | | | | |
| PCB-74 | Triglyceride levels | Lowest levels are observed in intermediate groups (quartile 2) | CARDIA participants (n = 90 controls from nested case-control study) | 590 |
| PCB-126 | Bone mineral density in right arm | With low exposures, fat mass had inverse associations with bone mineral density; with high exposures, fat mass had positive associations with bone mineral density | NHANES participants, aged <50 (n = 710 women, n = 768 men) | 835 |
| PCB-138 | Bone mineral density in right arm | With low exposures, fat mass had inverse associations with bone mineral density; with high exposures, fat mass had positive associations with bone mineral density | NHANES participants, women aged 50+ (n = 679 women, n = 612 men) | 835 |
| PCB-153 | Telomere length in peripheral leukocytes | Increased length with intermediate exposure groups (quintile 4) | Adults aged 40+ (Korea, n = 84) | 591 |
| PCB-170 | Diabetes incidence | Highest risk in groups with intermediate exposures (quartile 2) | CARDIA participants, case-control study (n = 90 cases and n = 90 controls) | 833 |
| | Endometriosis | Decreased risk in groups with intermediate exposures (quartile 3) | Participants from the Women at Risk of Endometriosis (WREN) study, 18–49 yr old, case-control study (n = 251 cases; n = 538 controls) | 838 |
| PCB-172 | DNA hypomethylation (by Alu assay) | Highest levels of hypomethylation in groups with lowest and highest exposures | Adults aged 40+ (Korea, n = 86) | 839 |
| PCB-180[a] | BMI | Highest BMI with intermediate exposures (quartile 2) | CARDIA participants (n = 90 controls from nested case control study) | 590 |
| PCB-187[a] | HDL cholesterol levels | Lowest levels with intermediate exposures (quartile 2) | CARDIA participants (n = 90 controls from nested case control study) | 590 |
| PCB 196–203 | Diabetes incidence | Highest risk in groups with intermediate exposures (quartile 2) | CARDIA participants, case-control study (n = 90 cases and n = 90 controls) | 833 |
| PCB-196 | Endometriosis | Decreased risk in groups with intermediate exposures (quartile 3) | Participants from the Women at Risk of Endometriosis (WREN) study, 18–49 yr old, case-control study (n = 251 cases; n = 538 controls) | 838 |

(*Continued*)

**TABLE 9.** Continued

| Chemicals by chemical class | Affected endpoint | NMDRC | Study subjects | Refs. |
|---|---|---|---|---|
| PCB-199[a] | Triglyceride levels | Highest risk in groups with intermediate exposures (quartiles 2–3) | CARDIA participants (n = 90 controls from nested case control study) | 590 |
| PCB-201 | Endometriosis | Decreased risk in groups with intermediate exposures (quartiles 2–3) | Participants from the Women at Risk of Endometriosis (WREN) study, 18–49 yr old, case-control study (n = 251 cases, n = 538 controls) | 838 |
| Heavy metals | | | | |
| Selenium | Fasting glucose levels (by modeled exposure) | Intermediate exposures have highest fasting glucose levels | NHANES 2003- 2004 participants, aged 40+ (n = 917) | 840 |
| | Glycosylated hemoglobin (by modeled exposure) | Intermediate exposures have highest % glycosylated hemoglobin | NHANES 2003- 2004 participants, aged 40+ (n = 917) | 840 |
| | Diabetes incidence (by modeled exposure) | Intermediate exposures have highest risk for diabetes | NHANES 2003- 2004 participants, aged 40+ (n = 917) | 840 |
| | Blood triglyceride levels | Intermediate exposures have highest triglyceride levels | NHANES participants, aged 40+ (n = 1159) | 841 |
| Arsenic | Cytokines in umbilical cord blood | Lower inflammatory markers at intermediate exposures (quartile 2) | Pregnant women in Bangladesh ( n = 130) | 842 |
| Manganese | Mental development scores in infants and toddlers | Intermediate exposures had highest mental development scores at 12 months of age; association lost in older toddlers | 12-month-old infants, Mexico (n = 301) | 843 |
| | Sperm count, motility and morphology | Intermediate doses had lowest sperm counts and motility; intermediate doses also had the worst sperm morphologies | Men aged 18–55 (infertility clinic patients, n = 200) | 844 |
| Mixtures | | | | |
| 31 POP | Diabetes incidence | Highest incidence in intermediate groups (sextiles 2–3) | CARDIA participants, case-control study (n = 90 cases and n = 90 controls) | 833 |
| 16 POP | Diabetes incidence | Highest incidence in intermediate groups (sextiles 2–3) | CARDIA participants, case-control study (n = 90 cases and n = 90 controls) | 833 |
| Non-dioxin-like PCB (mix) | Metabolic syndrome | Highest incidence in intermediate groups (quartile 3) | NHANES 1999–2002 participants, aged 20+ (n = 721) | 845 |
| Dioxin-like PCB (mix) | Triacylglycerol levels by quartile of exposure | Highest levels in intermediate groups (quartile 3) | NHANES 1999–2002 participants, aged 20+ (n = 721) | 845 |
| **Additional supportive evidence for NMDRC in the epidemiology literature** | | | | |
| Insecticides | | | | |
| Heptachlor epoxide | Prevalence of newly diagnosed hypertension | Highest risk in intermediate groups (quartile 2); other endpoints do not have NMDRC | NHANES participants, women aged 40+, cross-sectional (n = 51 cases, n = 278 total) | 826 |
| $\beta$-Hexachloro-cyclohexane | Triacylglycerol levels by quartile of exposure | Highest risk in intermediate group (quartile 2) | NHANES participants, aged 20+ (n = 896 men, 175 with metabolic syndrome) | 845 |
| Plastics | | | | |
| Mono-*N*-butyl phthalate (MBP) | BMI, age-specific effects | Effects seen only in elderly participants (age 60–80); risk is lowest in quartile 3 | NHANES male participants (n = 365; age 60–80) | 470 |
| Mono-benzyl phthalate (MBzP) | BMI, age-specific effects | Effects seen only in young participants (age 6–11); risk is highest in quartiles 2–3 | NHANES participants (both sexes, n = 329 males; n = 327 females) | 470 |
| Flame retardants | | | | |
| PFOA | Thyroid disease (self-reported) | Lowest risk in intermediate groups (quartile 3) | NHANES 1999–2000, 2003–2006 participants, males aged 20+ (n = 3974) | 837 |
| Dioxin and related compounds | | | | |
| TCDD | Age at natural menopause | Highest for intermediate exposure group (quintile 4) | Highly exposed women; Seveso Women's Health Study participants (n = 616) | 468 |
| HCDD | Bone mineral density in right arm by quintile of fat mass | With low exposures, fat mass had inverse associations with bone mineral density; with high exposures, fat mass had positive associations with bone mineral density | NHANES participants, women aged 50+ (n = 679 women, n = 612 men) | 835 |
| Heavy metals | | | | |
| Selenium | Prevalence of peripheral artery disease | Disease prevalence decreased in intermediate doses, then increased gradually with higher doses | NHANES participants, aged 40+ (n = 2062) | 469 |

BMI, Body mass index; HCDD, hexachloro-dibenzo-p-dioxin; HDL, high-density lipoprotein. PCB, polychlorinated biphenyls; PFOA, perfluorooctanoic acid; PBB, polybrominated biphenyl; PBDE, polybrominated diphenyl ethers; POP, persistent organic pollutants

[a] In many cases, multiple chemicals in the same class had similar effects. A few chemicals were selected to illustrate the observed effect. This list is not comprehensive.

organs, or animal models over limited periods of time. Causal inference is not done directly from the epidemiological study results; instead, it is done via combining information from the epidemiological observations with findings from the detailed studies of pathways and animals.

We have suggested that NMDRCs are a fundamental and general feature of hormone action in cells and animals.

**Figure 5.**

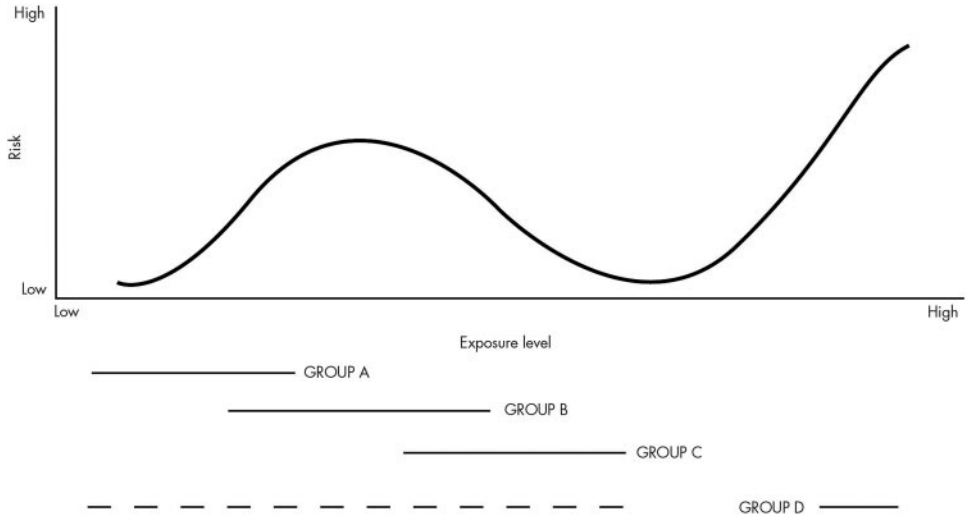



Figure 5. Example of a NMDRC in humans and the sampling populations that could be examined in epidemiology studies. This schematic illustrates a theoretical NMDRC in a human population. If a study were to sample only group A, the conclusion would be that with increasing exposures, risk increases monotonically. Sampling group B would allow researchers to conclude that there is a nonmonotonic relationship between exposure level and risk. If a study included only group C, the conclusion would be that with increasing exposures, there is decreased risk of disease. Group D represents a population that was highly exposed, *i.e.* due to an industrial accident. This group has the highest risk, and there is a monotonic relationship between exposures and risk, although risk is high for all individuals. In the group D situation, there is generally a background population with which high-dose exposure is compared (*dotted line*); relative risk for group D would depend on whether that background population resembles group A, B, or C. From this example, it is clear that the population sampled could strongly influence the shape of the dose-response curve produced as well as the conclusions reached by the study.

It is therefore worth asking whether NMDRCs are expected in the epidemiology literature. The endpoints assessed in epidemiology studies are typically integrated effects, rather than short-term effects; therefore, the various cell- or organ-specific effects may cancel each other, particularly if they are NMDRCs (because they are unlikely to all have nonmonotonicity at the same dose and direction). Thus, NMDRCs are likely to be rarer in the epidemiology literature compared with studies examining the effects of a wide range of doses of an EDC on animals and cultured cells. Yet it is also important to ask what can be concluded if a NMDRC is detected in one epidemiology study but not in others examining the same chemical and outcome. There are several factors that must be considered. The first is that differences in the populations examined between the two studies could explain why a monotonic relationship is observed in one group and a nonmonotonic relationship in another (see Fig. 5). The second is that one or more studies may not be statistically designed to detect NMDRCs. Finally, it is plausible that the NMDRC is an artifact due to residual confounding or some other factor that was not considered in the experimental design. As more becomes known about the mechanisms operating in cells, tissues, and organs to generate NMDRCs, our ability to apply this information to epidemiology studies will increase as well.

### *4. Tamoxifen flare, a NMDRC observed in cells, animals, and human patients*

Although there is controversy in toxicology and risk assessment for endocrine disruptors, NMDRCs are recognized and used in current human clinical practice, although under a different specific term, flare. Flare is often reported in the therapy of hormone-dependent cancers such as breast and prostate cancer. Clinically, failure to recognize the NMDRC that is termed a flare would be considered malpractice in human medicine.

Tamoxifen flare was described and named as a transient worsening of the symptoms of advanced breast cancer, particularly metastases to bone associated with increased pain, seen shortly after the initiation of therapy in some patients (543). If the therapy could be continued, the patients showing tamoxifen flare demonstrated a very high likelihood of subsequent response to tamoxifen, including arrest of tumor growth and progression of symptoms for some time.

The subsequent mechanism of the flare was described in basic lab studies in athymic mouse models of human hormone-dependent breast cancer xenografts (544) and in tissue culture of hormone-dependent human breast cancer cells (545–547). In these models, it was observed that although high, therapeutic concentrations of tamoxifen inhibited estrogen-stimulated proliferation of breast cancer cells, lower concentrations of tamoxifen actually stimulated breast can-

**Figure 6.**

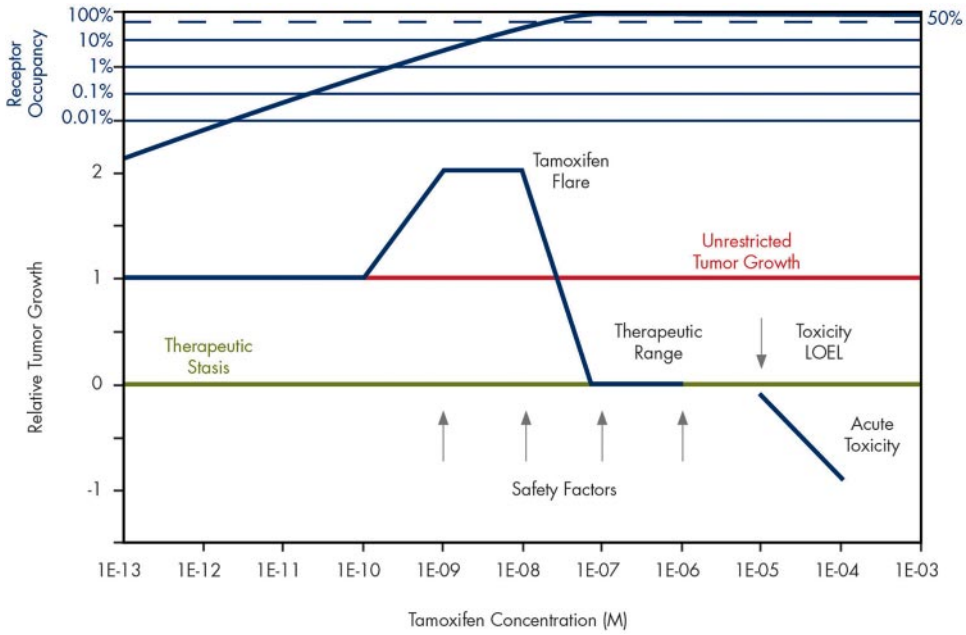



Figure 6. Dose-response ranges for tamoxifen in breast cancer therapy. This figure demonstrates the NMDRC, also called flare, in tamoxifen treatments. As the circulating dose of tamoxifen increases when treatment starts, patients initially experience flare, *i.e.* growth of the tumor (546), followed by a decrease in tumor size as the circulating levels of tamoxifen rise into the therapeutic range (676, 677). High doses of tamoxifen are acutely toxic (546). Starting from the highest concentrations, where acute toxicity is observed, and going to lower concentrations on the X-axis, the acute toxicity diminishes towards zero growth, *i.e.* therapeutic stasis (*green* baseline). This occurs at approximately 1E-05 m, the lowest observed effect level (LOEL) for toxicity. The vertical arrows show the results of applying three or four 10-fold safety factors to the LOEL for the high-dose toxicity of tamoxifen, and would calculate a safe or reference dose for tamoxifen in the region of flare, the least safe region of exposure in actual practice. Above the diagram of dose response ranges is estimated ER occupancy by tamoxifen. This was calculated from the affinity constant of tamoxifen for ERs determined in human breast cancer cells (Ki = 29.1 nM; Ref 678); flare appears to correspond to low receptor occupancy (*blue axis*), therapeutic range with mid and upper-range receptor occupancy, and acute toxicity well above 99% receptor occupancy. (678).

cer cell growth as long as the cells were estrogen dependent (548). Tamoxifen was also shown to disrupt tissue organization of the mammary gland, with specific effects on the stroma that may contribute to the observed effects on proliferation of epithelial cells (549, 550).

Tamoxifen therapy is administered as 10 mg twice per day (20 mg/d; approx 0.3 mg/kg body weight per day), but the target circulating levels are in the near submicromolar range (0.2–0.6 $\mu$M); these levels are reached slowly, after approximately 2 wks of therapy (551). In the initial period, where tamoxifen flare is observed, the circulating concentrations are ascending through lower concentrations, in the range below therapeutic suppression of growth, where breast cancer cell proliferation is actually stimulated by the drug, both in tissue culture, in animal xenograft studies, and in human patients (reviewed in Ref. 548). The recognition of this dual dose-response range for tamoxifen (low-dose, low-concentration estrogenic growth-stimulatory and higher-dose, higher-concentration estrogenic growth-inhibitory responses) led to the definition of the term selective estrogen response modulator, or SERM, activity (552–554). This SERM activity has since been observed for many or even most estrogenic EDCs, including BPA (3, 555–557).

These observations defined three separate dose-response ranges for the SERM tamoxifen in human clinical use. The lowest dose-response range, the range of flare, stimulated breast cancer growth and symptoms in some patients with hormone-dependent cancer. The next higher dose-response range is the therapeutic range where tamoxifen inhibits estrogen-dependent tumor growth. The highest dose range causes acute toxicity by the SERM (see Fig. 6).

Tamoxifen provides an excellent example for how high-dose testing cannot be used to predict the effects of low doses. For tamoxifen (as for other drugs), the range of acute human toxicity for tamoxifen was determined in phase I clinical trials. Phase I trials also defined an initial therapeutic range, the second dose-response range, as a dose below which acute toxicity was not observed. The therapeutic dose range was tested and further defined in phase II and later clinical trials to determine efficacy (see for example Ref. 558). Standard toxicological testing from

**Figure 7.**

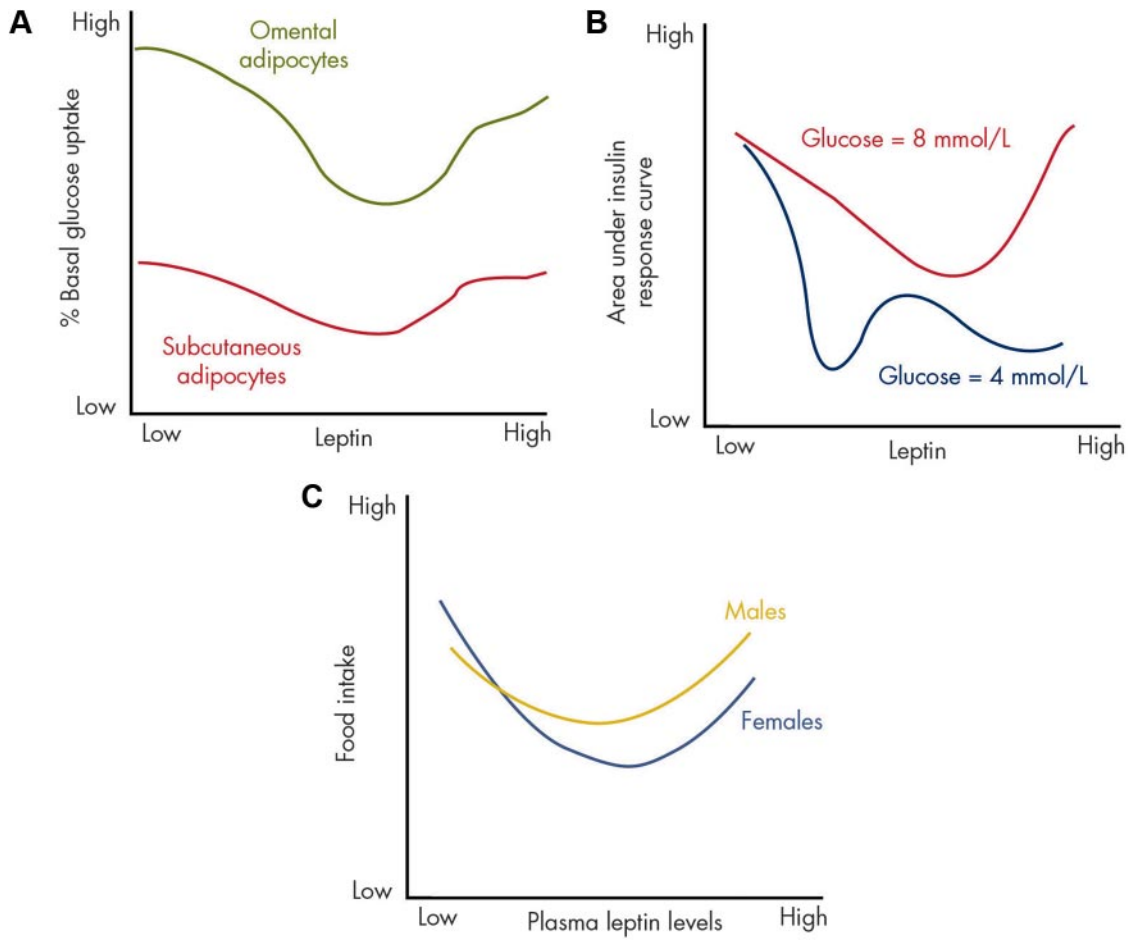



Figure 7. Leptin as an example of a NMDRC. Several studies report NMDRCs in response to leptin treatments. A, NMDRCs are observed in cultured primary adipocytes after leptin exposure. This graph illustrates the relationship between administered leptin dose and glucose uptake in two types of adipocytes, those isolated from omental tissue (*green*) and others from sc fat (*purple*) (schematic was made from data in Ref. 559). These data are on a log-linear plot. B, *Ex vivo* rat pancreas was treated with leptin and various doses of glucose, and the insulin response curves were examined. Area under the curve is a measure of the ability of the pancreas to bring glucose levels under control. Different dose-response curves were observed depending on the amount of glucose administered: a U-shaped curve when 8 mmol/liter was included (*pink*) or a multiphasic curve with 4 mmol/liter (*blue*) (schematic made from data in Ref. 560). These data are on a linear-linear plot. C, U-shaped NMDRCs were also observed when food intake was compared with leptin levels in the blood of rats administered the hormone. This response was similar in males (*orange*) and females (*cyan*) (schematic made from data in Ref. 562). These data are on a linear-linear plot.

high doses to define a LOAEL or NOAEL are equivalent to the phase I clinical testing, and in risk assessment, a safe dose or reference dose is calculated from these tests. However, the lowest dose range, with the highly adverse effects termed flare, was not detected in the phase I trials and was determined only for tamoxifen in breast cancer therapy at the therapeutic doses (543). The implication for risk assessment is that NMDRCs for EDCs, particularly those already identified as SERMs, would likely not be detected by standard toxicological testing at high doses. That is, the consequence of high-dose testing is the calculation of a defined but otherwise untested safe dose that is well within the range equivalent to flare, *i.e.* a manifestly unsafe dose of the EDC (Fig. 6).

### *5. Similarities in endpoints across cell culture, animal, and epidemiology studies: evidence for common mechanisms?*

There are common trends in some findings of NMDRCs in cell, animal, and human studies and therefore evidence for related mechanisms for NMDRCs at various levels of biological complexity. Tamoxifen flare, discussed in *Section III.C.4*, is an informative example. Another illustrative example is that of the effect of the hormone leptin (Fig. 7). In cultured primary adipocytes, NMDRCs are observed after leptin exposure; moderate doses of leptin significantly reduce insulin-mediated glucose intake, whereas low and high doses maintain higher glucose intake in response to insulin (559). The rat pancreas shows a similar response to leptin; the amount of

secreted insulin has an inverted U-shaped response to leptin (560, 561). Even more striking is the relationship between leptin and food intake. Rats administered moderate doses of leptin consume less food compared to rats dosed with low or high levels of leptin (562); mechanistically, this lower food intake could be due to higher circulating glucose levels in these animals due to ineffective insulin action. And finally, in a human study, leptin levels were found to correlate with body mass index but have a U-shaped relationship with mortality (563). These results suggest that hormones can produce similar responses at several levels of biological complexity (cell, organ, animal, and population).

A large number of epidemiology studies with NMDRCs have found relationships between EDC exposures like POPs and metabolic diseases including obesity and diabetes (Table 9) (see also Ref. 564 for a review), and the mechanisms for these relationships have begun to be explored. Human and animal cells treated with EDCs in culture display NMDRCs that are relevant to these diseases: BPA has nonmonotonic effects on the expression of adipocyte proteins in preadipocytes and the release of adiponectin from mature adipocytes (565–567). Similarly, in female rodents, low doses but not high doses of BPA increased adipose tissue weight and serum leptin concentrations (568), and intermediate doses of phthalates decrease serum cholesterol levels (569). Thus, although understanding the mechanisms operating at the cellular level of organization has not yet led to definitive knowledge of the mechanisms producing NMDRCs in human populations, there appear to be strong similarities in cells, animals, and humans that support a call for continued work focusing on metabolic disease endpoints at each level of biological organization.

### D. NMDRC summary

We have demonstrated that nonmonotonicity is a common occurrence after exposures to hormones and EDCs in cell culture and animals and across human populations. Because of the abundance of examples of NMDRCs, we expect that if adequate dose ranges are included in animal and cell culture studies, including the use of negative and well-chosen positive controls, NMDRCs may be observed more often than not. Here, we have focused mainly on studies that examined a wide range of doses, including many that examined the effects of doses that span the low-dose and toxicological ranges. We also discussed several mechanisms that produce NMDRCs. Each of these mechanisms can and does operate at the same time in a biological system, and this cooperative action is ultimately responsible for NMDRCs.

Understanding nonmonotonicity has both theoretical and practical relevance. When a chemical produces monotonic responses, all doses are expected to produce similar effects whose magnitude varies with the dose, but when a chemical produces a NMDRC, dissimilar or even opposite effects will be observed at different doses. Thus, monotonic responses can be modeled using the assumption that each step in a linear pathway behaves according to the law of mass action (43, 570); high doses are always expected to produce higher responses. In contrast, NMDRCs are not easy to model (although they are quite easy to test for), requiring detailed knowledge of the specific mechanisms operating in several biological components. From a regulatory standpoint, information from high doses cannot always be used to assess whether low doses will produce a biological effect (38).

## IV. Implications of Low-Dose Effects and Nonmonotonicity

Both low-dose effects and NMDRCs have been observed for a wide variety of EDCs as well as natural hormones. Importantly, these phenomena encompass every level of biological organization, from gene expression, hormone production, and cell number to changes in tissue architecture to behavior and population-based disease risks. One conclusion from this review is that low-dose effects and NMDRCs are often observed after administration of environmentally relevant doses of EDCs. For both hormones and EDCs, NMDRCs should be the default assumption absent sufficient data to indicate otherwise. Furthermore, there are well-understood mechanisms to explain how low-dose effects and NMDRCs manifest *in vitro* and *in vivo*. Accepting these phenomena, therefore, should lead to paradigm shifts in toxicological studies and will likely also have lasting effects on regulatory science. Some of these aspects are discussed below. Additionally, we have briefly explored how this knowledge should influence future approaches in human and environmental health.

At a very practical level, we recommend that researchers publishing data with low-dose and nonmonotonic effects include key words in the abstract/article that identify them as such specifically. This review was unquestionably impeded because this has not been standard practice. We also strongly recommend that data showing nonmonotonic and binary response patterns not be rejected or criticized because there is no dose response.

### A. Experimental design

#### *1. Dose ranges must be chosen carefully*

To detect low-dose effects or NMDRCs, the doses included for testing are of utmost importance. Most of the studies we examined here for nonmonotonicity tested

doses over severalfold concentrations. Unfortunately, regulatory guidelines only require that three doses be tested. Both low-dose effects and NMDRCs can be observed when examining only a few doses, but some studies may detect significant results purely by luck, because a small shift in dose can have a large impact on the ability to observe differences relative to untreated controls.

In the multitude of chemicals that have never been tested at low doses, or in the development of new chemicals, to determine whether a chemical has low-dose effects in laboratory animals, we suggest setting the NOAEL or LOAEL from traditional toxicological studies as the highest dose in experiments specifically designed to test endocrine-sensitive endpoints. We suggest setting the lowest dose in the experiment below the range of human exposures, if such a dose is known. Several intermediate doses overlapping the range of typical human exposures should be included also, bringing the total number in the range of five to eight total doses tested. Importantly, although the levels of many environmental chemicals in human blood and/or urine have been reported by the CDC and other groups responsible for population-scale biomonitoring, it is often not known what administered doses are needed to achieve these internal exposure levels in animals (4, 253); thus, toxicokinetic studies are often needed before the onset of low-dose testing. This is important because the critical issue is to determine what effects are observed in animals when circulating levels of an EDC match what is measured in the typical human. Due to differences in metabolism, route of exposure, and other factors, a relatively high dose may need to be administered to a rodent to produce blood concentrations in the range of human levels; however, this should not be considered a high-dose study.

It has also been suggested that animal studies that are used to understand the potential effects of a chemical on humans should use a relevant route of administration to recapitulate human exposures (571, 572) because there may be differences in metabolism after oral and nonoral administration. Many chemicals that enter the body orally undergo first-pass metabolism and are then inactivated via liver enzymes, whereas other routes (*i.e.* sc) can bypass these mechanisms and lead to a higher concentration of the active compound in circulation (573). Studies indicate, however, that inactivation of chemicals via first-pass metabolism is not complete and also that deconjugation of metabolites can occur in some tissues allowing the re-release of the active form (574, 575). Additionally, for some chemicals, it is clear that route of administration has little or no impact on the availability of the active compound in the body (241, 384), and other studies show that route of administration has no impact on the biological effects of these chemicals; *i.e.* regardless of how it enters the body, dioxin has similar effects on exposed individuals (384), and comparable results have been observed for BPA (141). Although understanding the typical route of human exposure to each environmental chemical is an important task, it has been argued that any method that leads to blood concentrations of a test chemical in the range they are observed in humans is an acceptable exposure protocol, and this is especially true with gestational exposures, because fetuses are exposed to chemicals only via their mothers' blood (31, 576).

#### *2. Timing of exposures is important*

Rodent studies indicate that EDC exposures during development have organizational effects, with permanent effects that can manifest even in late adulthood, whereas exposures after puberty are for the most part activational, with effects that are abrogated when exposures cease. For example, the adult uterus requires relatively large doses of BPA (in the parts-per-million range) to induce changes associated with the uterotrophic assay (555, 577), whereas parts-per-trillion and ppb exposures during the fetal period permanently and effectively alter development of the uterus (279, 310, 578). Thus, the timing of exposures is profoundly important to detect low-dose effects of EDCs.

Human studies also support this conclusion. The 1976 explosion of a chemical plant in Seveso, Italy, which led to widespread human exposure to large amounts of TCDD, a particularly toxic form of dioxin, and the deposition of this chemical on the land surrounding the chemical plant, provided evidence in support of the organizational and activational effects of endocrine-active chemicals in humans (579). Serum TCDD concentrations showed correlations between exposure levels and several disease outcomes including breast cancer risk, abnormal menstrual cycles, and endometriosis (580–582), but individuals who were either infants or teenagers at the time of the explosion were found to be at greatest risk for developing adult diseases (583, 584). Importantly, many scientists have argued that organizational effects can occur during puberty, *i.e.* that the period where hormones have irreversible effects on organ development extends beyond the fetal and neonatal period (585), and for some endpoints this appears to be the case (586, 587).

It has also been proposed that the endocrine system maintains homeostasis in the face of environmental insults (210). The adult endocrine system does appear to provide some ability to maintain a type of homeostasis; when the pharmaceutical estrogen DES is administered to pregnant mice, the circulating estradiol concentrations in the dam respond by decreasing linearly (224). In contrast, fetal concentrations of estradiol respond nonmonotonically in

a way that is clearly not correlated with maternal levels. Similarly, there is evidence that BPA can induce aromatase and therefore increase estradiol levels *in situ* in the fetal urogenital sinus (588). This is an example of a feed-forward positive-feedback effect rather than a homeostatic response. The effects of EDCs on adult subjects, both animal and people, suggest that diseases often result from low-dose adult exposures (589–595); this argues against a view of the endocrine system as a means to maintain homeostatic control. Instead, individuals can be permanently changed, in an adverse way, after EDC exposures.

In one example, pregnant mice were exposed to low concentrations of BPA, and their male offspring had altered pancreatic function at 6 months of age (158). Surprisingly, however, the mothers (exposed only during pregnancy) were also affected, with altered metabolic machinery and body weight at 4 months postpartum, long after exposures had ended. The increased incidence of breast cancer in women that took DES during pregnancy also illustrates this point (596, 597). These studies suggest that even the adult endocrine system is not invariably capable of maintaining a so-called homeostatic state when exogenous chemicals affecting the endocrine system are present. Thus, although adult exposures to EDCs have been given some attention by bench scientists (29), more work of this kind is needed to better understand whether and how EDCs can have permanent organizational effects on adult animals.

At the beginning of this review, we justified the need to critically examine the low-dose literature because of recent epidemiological findings linking EDC exposures and diseases. Yet there is inherent difficulty in examining neonatal exposures to EDCs and their connection to diseases due to the length of time needed for these studies; thus, many studies of this type have examined high doses of pharmaceuticals (*i.e.* DES) or accidental exposures to industrial chemicals (*i.e.* dioxin) (66, 398, 399, 581, 597–601).

Only recently, with the availability of biomonitoring samples from large reference populations, have lower doses begun to receive widespread attention from epidemiologists. Many recent studies have examined adult exposures to EDCs and correlated exposures with disease statuses (see for example Refs. 15, 16, and 602–604). Human studies examining fetal/neonatal exposures to low-dose EDCs and early life effects have also begun to be studied (6, 333, 605–607), although studies linking these early life exposures to adult diseases are likely to be decades away. More than anything, these studies support our view that the effects of low-dose exposures should be considered when determining chemical safety.

#### *3. Importance of endpoints being examined*

Traditional toxicology testing, and in particular those studies performed for the purposes of risk assessment, typically adhere to guideline studies that have been approved by international committees of experts (608). The endpoints assessed in these guideline-compliant studies are centered around higher-order levels, including death, weight loss, mortality, and changes in organ weight, and a limited number of histopathological analyses (609, 610). When pregnant animals are included in toxicological assessments, the endpoints measured typically include the ability to maintain pregnancies, the number of offspring delivered, sex ratios of surviving pups, and measures regarding maternal weight gain and food/water intake (610).

Yet low-dose EDCs are rarely toxic to the point of killing adult animals or causing spontaneous abortions, and traditional tests such as the uterotrophic assay have been shown to be relatively insensitive (72, 577). It has been argued that this type of testing is insufficient for understanding the effects of EDCs (31, 70, 495, 611). Many EDC studies have instead focused on examining newly developed, highly sensitive endpoints that span multiple levels of biological organization, from gene expression to tissue organization to organ systems to the whole animal (612), which may not be rapidly lethal but which nonetheless have enormous importance for health, including mortality. Thus, for example, studies designed to examine the effects of chemicals on obesity no longer focus on body weight alone but also analyze gene expression; fat content in adipose cells and the process of adipogenesis; inflammation, innvervation, and vascularization parameters in specific fat pads; conversion rates of white and brown adipose tissues; systemic hormone levels and response to glucose and insulin challenges; and food intake and energy expenditures, among others (314, 613–615). As our knowledge of EDCs and the endocrine system continue to grow, the most sensitive endpoints should be used to determine whether a chemical is disrupting the development of organisms (70).

In moving beyond traditional, well-characterized health-related endpoints like mortality and weight loss, an important question has been raised: how do we define endpoints as adverse? This is an important point, because it has been suggested that the creative endpoints examined in independent EDC studies are not validated and may not represent adverse effects (609). There is also debate over whether the mechanism (or mode) of action must be explained for each effect to determine whether a relevant pathway is present in humans (616, 617). Yet, when originally assessing the low-dose literature, the NTP expert panel chose to examine all effects of EDC exposure, re-

gardless of whether the endpoint could be deemed adverse (2). From the perspective of developmental biology, any change in development should be seen as adverse, even if the change itself is not associated with a disease or dysfunction. Some of these developmental changes, in fact, may increase sensitivity or susceptibility to disease later on in life but will otherwise appear normal. Furthermore, studies of heavy metals have shown that small shifts in parameters like IQ may not have drastic effects on individuals but can have serious repercussions on the population level (618), and therefore changes in the variance/observable range of a phenotype should also be considered adverse (52).

#### *4. Importance of study size*

National Institutes of Health guidelines require that the number of vertebrate animals used in experiments be as small as possible to show statistically significant effects based on power analysis. Yet many traditional toxicology studies have used large numbers of animals to draw conclusions about chemical safety. When the endpoints being assessed have binary outcomes (*i.e.* animal has a tumor *vs.* animal does not have a tumor) and the incidence of the phenotype is not high, a large number of animals is required to reveal statistically significant effects. In contrast, many of the endpoints examined in the field of endocrine disruption are more complex and are not binary; thus, power analysis allows researchers to determine how many animals are needed to observe statistically significant (and biologically relevant) differences between control and exposed populations. For this reason, arbitrary numbers set as cutoffs for determining whether a study is acceptable or unacceptable for risk assessments are not appropriate. Instead, the number of animals required for a study to be complete is dependent on the effect size, precision/variance, minimal meaningful difference to be considered between populations, and the $\alpha$-value set in statistical tests.

### B. Regulatory science

For decades, regulatory agencies have tested, or approved testing, of chemicals by examining high doses and then extrapolating down from the NOAEL, NOEL, and LOAEL to determine safe levels for humans and/or wildlife. As discussed earlier, these extrapolations use safety factors that acknowledge differences between humans and animals, exposures of vulnerable populations, interspecies variability, and other uncertainty factors. These safety factors are informed guesses, not quantitatively based calculations. Using this traditional way of setting safe doses, the levels declared safe are never in fact tested. Doses in the range of human exposures are therefore also unlikely to be tested. This has generated the current state of science, where many chemicals of concern have never been examined at environmentally relevant low doses (see Table 4 for a small number of examples).

Assumptions used in chemical risk assessments to estimate a threshold dose below which daily exposure to a chemical is estimated to be safe are false for EDCs. First, experimental data provide evidence for the lack of a threshold for EDCs (619). More broadly, the data in this review demonstrate that the central assumption underlying the use of high doses to predict low-dose effects will lead to false estimates of safety. The use of only a few high doses is based on the assumption that all dose-response relationships are monotonic and therefore that it is appropriate to apply a log-linear extrapolation from high-dose testing to estimate a safe reference dose (Fig. 4). The Endocrine Society issued a position statement on EDCs (620) and urged the risk assessment community to use the expertise of their members to develop new approaches to chemical risk assessments for EDCs based on principles of endocrinology. Undertaking this mission will represent a true paradigm shift in regulatory toxicology (79). The Endocrine Society statement was then supported in March 2011 by a letter to *Science* from eight societies with relevant expertise representing over 40,000 scientists and medical professionals (621).

Studies conducted for the purposes of risk assessment are expected to include three doses: a dose that has no effects on traditional toxicological endpoints (the NOAEL), a higher dose with effects on traditional endpoints (the LOAEL), and an even higher dose that shows toxicity. Although reducing the number of animals used for these types of studies is an important goal, more than three doses are often needed for a true picture of a chemical's toxicity. The examination of a larger number of doses would allow for 1) the study of chemicals at the reference dose, *i.e.* the dose that is calculated to be safe; 2) examination of doses in the range of actual human exposures, which is likely to be below the reference dose; and 3) the ability to detect NMDRCs, particularly in the low-dose range. The impact of testing more doses on the numbers of animals required can be mitigated by use of power analysis, as suggested above. Because no amount of research will ever match the diversity and reality of actual human experience, there should be ongoing epidemiological study of potential adverse effects of EDCs even after safe levels are published, with periodic reevaluation of those safe levels.

One issue that has been raised by regulatory agencies is whether animal models are appropriate for understanding the effects of EDCs on humans. These arguments largely center around observed differences in hormone levels during different physiological periods in rodents and humans (57), and differences in the metabolic machinery and ex-

cretion of chemicals between species (622). To address the first issue, it should be noted that the FDA uses animals to test pharmaceuticals and other chemicals before any safety testing in humans because it is widely recognized that, although animals and humans do not have exactly the same physiologies, there is evolutionary conservation among vertebrates and specifically among mammals (62). Furthermore, animal studies proved to be highly predictive of the effects of DES on women, indicating that rodents are sufficiently similar to humans to reliably forecast affected endpoints in the endocrine system (64, 623). Thus, the default position must be that animal data are indicative of human effects until proven otherwise.

With regard to the second issue, BPA researchers in particular have examined species-specific differences in metabolism of this EDC. Interestingly, the pharmacokinetics of BPA in rodents, monkeys, and humans appear to be very similar (624), and regulatory agencies have subsequently concluded that rodents are appropriate models to assess the effects of this chemical (625, 626). Thus, researchers should select animal models that are sensitive to low doses of hormones and select appropriate species for the endpoints of interest. As the scope of our knowledge has broadened about how chemicals can alter the endocrine system, well beyond estrogens, androgens, and the thyroid, it is imperative that considerable thought be given to how to apply this for regulatory purposes.

### C. Human health

As discussed several times throughout this review, there is now substantial evidence that low doses of EDCs have adverse effects on human health. Thus, although many epidemiological studies originally focused on occupationally exposed individuals and individuals affected by accidental exposures to high doses of environmental chemicals, these recent studies have suggested wide-ranging effects of EDCs on the general population.

Importantly, human exposures are examples of true mixtures; dozens if not hundreds of environmental chemicals are regularly detected in human tissues and fluids (91), yet very little is known about how these chemicals act in combination (627). Several studies indicate that EDCs can have additive or even synergistic effects (143, 323, 628–630), and thus these mixtures are likely to have unexpected and unpredictable effects on animals and humans. The study of mixtures is a growing and complex field that will require considerable attention in the years ahead as knowledge of EDCs in the laboratory setting are applied to human populations (631, 632).

How much will human health improve by testing chemicals at low, environmentally relevant doses and using the results to guide safety determinations? Current testing paradigms are missing important, sensitive endpoints; because they are often unable to detect NMDRCs, they cannot make appropriate predictions about what effects are occurring at low doses. At this time, it is not possible to quantify the total costs of low-dose exposures to EDCs. However, current epidemiology studies linking low-dose EDC exposures to a myriad of health problems, diseases, and disorders suggest that the costs of current low-dose exposures are likely to be substantial.

The weight of the available evidence suggests that EDCs affect a wide range of human health endpoints that manifest at different stages of life, from neonatal and infant periods to the aging adult. As the American population ages, healthcare costs continue to rise, and there are societal costs as well, with decreased quality of life concerns, decreases in work productivity due to illness or the need for workers to care for affected family members, and the psychological stresses of dealing with some outcomes like infertility. Thus, it is logical to conclude that low-dose testing, followed by regulatory action to minimize or eliminate human exposures to EDCs, could significantly benefit human health. This proposal effectively calls for greatly expanded research to give human communities feedback about themselves. It emanates from a view that human society benefits greatly from the many chemical compounds it uses but that extensive epidemiological surveillance and other focused research designs are needed to assure that the balance of risk/benefit from those chemicals is acceptable.

How much would human health benefit by a reduction in the use of EDCs? For some chemicals, minor changes in consumer habits or industrial practices can have drastic effects on exposures (633–636). Other chemicals like DDT that have been regulated in the United States for decades continue to be detected in human and environmental samples; the persistent nature of many of these agents suggests they may impact human health for decades to come. Even less-persistent chemicals like BPA are likely to remain in our environment long after a ban is enacted because of the large amounts of plastic waste leaching BPA (and other estrogenic compounds) from landfills into water sources (637) and its presence on thermal receipt paper and from there into recycled paper (638–640). Yet, despite these challenges, reducing human exposure to EDCs should be a priority, and one way to address that priority is to decrease the production and use of these chemicals. The Endocrine Society has called for such a reduction and the use of the precautionary principle, *i.e.* action in the presence of concerning information but in the absence of certainty to eliminate or cut the use of questionable chemicals even when cause-effect relationships are not yet established (620).

### D. Wildlife

Much of the recent focus on EDCs has been on the impact of these chemicals on human health. Yet the earliest studies of EDCs that focused on the impact of these chemicals on wildlife should not be forgotten. Rachel Carson's work on DDT and other pesticides provided some of the earliest warning signs that there were unintended consequences of chemical use. Carson's work was ahead of its time; she understood that exceedingly small doses of these chemicals produced adverse effects, that the timing of exposures was critical, and that chemical mixtures produced compounded effects (641). Now, decades after some of the most dangerous EDCs have been regulated, they continue to be measured in environmental samples as well as the bodies of wildlife animals.

Furthermore, it should be pointed out that humans, like wildlife, are not insulated from the environment, and effects in wildlife, including nonmammalian species, are indicative of and mirror effects in humans. For example, BPA has estrogen-like effects in fish (642–644), amphibians (645, 646), and reptiles (647, 648). A recent review showed that demasculinizing and feminizing effects of atrazine have been demonstrated in fish, amphibians, reptiles, birds, and mammals, *i.e.* every vertebrate class examined (326); and in fact, the first report to suggest that atrazine induced aromatase was conducted in reptiles (649). Similarly, perchlorate affects fish (650–653), amphibians (654–658), and birds (659–661) via mechanisms consistent with those described for humans, and some of the earliest reports on perchlorate's effects on thyroid function were conducted in amphibians (661, 662). Finally, ecological studies of dioxin and dioxin-like chemicals reveal effects on a range of exposed wildlife including birds (663, 664), fish (665, 666), and invertebrates (667). Although these studies have highlighted some of the species-specific effects of dioxin (389), and orders of magnitude differences in toxic equivalency factors between species (668), they also indicate the conservation of mechanisms for the effects of dioxin on a range of biological endpoints in wildlife, laboratory animals, and humans (384). In fact, in many cases, nonmammalian species are much more sensitive to EDC effects, and wildlife species serve as sentinels for environmental and public health (669–673). Thus, the effects of these chemicals on wildlife populations are likely to continue; for this reason, the low-dose effects of these chemicals are particularly worth understanding (674, 675).

## V. Summary

In conclusion, we have provided hundreds of examples that clearly show that NMDRCs and low-dose effects are common in studies of hormones and EDCs. We have examined each of these issues separately and provided mechanistic explanations and examples of both. These topics are related, but they must be examined individually to be understood. The concept of nonmonotonicity is an essential one for the field of environmental health science because when NMDRCs occur, the effects of low doses cannot be predicted by the effects observed at high doses. In addition, the finding that chemicals have adverse effects on animals and humans in the range of environmental exposures clearly indicates that low doses cannot be ignored.

In closing, we encourage scientists and journal editors to publish data demonstrating NMDRCs and low-dose effects, even if the exact mechanism of action has not yet been elucidated. This is important because the study of EDC is a growing specialty that crosses many scientific fields, and scientists that work on or regulate EDCs should appreciate and acknowledge the existence of NMDRCs and low-dose effects and have access to this important information. We further recommend greatly expanded and generalized safety testing and surveillance to detect potential adverse effects of this broad class of chemicals. Before new chemicals are developed, a wider range of doses, extending into the low-dose range, should be fully tested. And finally, we envision that the concepts and empirical results we have presented in this paper will lead to many more collaborations among research scientists in academic and government laboratories across the globe, that more and more sophisticated study designs will emerge, that what we have produced herein will facilitate those making regulatory decisions, that actions taken in light of this information will begin to abate the use of EDCs, and ultimately that health impacts in people and in wildlife will be averted.

## Acknowledgments

We thank many colleagues in the fields of endocrine disruption and environmental health science for fruitful discussions on the topics covered in this manuscript. We also thank three anonymous reviewers whose comments and suggestions greatly improved this review.

Address requests for reprints to: Laura N. Vandenberg, Tufts University, Center for Regenerative and Developmental Biology, 200 Boston Avenue, Suite 4600, Medford, Massachusetts 02155. E-mail: laura.vandenberg@tufts.edu; or J. P. Myers, Environmental Health Sciences, 421 Park Street, Charlottesville, Virginia 22902. E-mail: jpmyers@ehsciences.org.

This work was supported by National Institutes of Health (NIH) Grants GM 087107 (to L.N.V.), ES 08314 (to A.M.S.), ES 010026 (to R.T.Z.), ES018764 (to F.S.v.S.), HL 53560 (to D.R.J.), UMC MO-VMFC0018 (to W.V.W.), a Susan G. Komen for Cure Grant FAS0703860 (to T.S.), grants from the Mitchell Kapor Foundation, the

Cornell-Douglas Foundation, and the Wallace Global Fund (to T.B.H.) and a grant from the Kendeda Foundation (to J.P.M.). This article may be the work product of an employee or group of employees of the National Institute of Environmental Health Sciences (NIEHS), NIH; however, the statements, opinions or conclusions contained therein do not necessarily represent the statements, opinions or conclusions of NIEHS, NIH, or the U.S. government.

We dedicate this manuscript to Professor Howard A. Bern. Dr. Bern was an exceptionally brilliant biologist and a generous and inspiring colleague. His work spanning a wide range of organisms addressed multiple aspects of organismal and evolutionary biology. He was one of the founders of the field of comparative endocrinology and a pioneer in the study of endocrine disruption, anticipating the deleterious effects of developmental exposure to estrogens one decade before the discovery of the effects of diethylstilbestrol in women fetally exposed to this chemical. His pioneering work included, among other subjects, neuroendocrinology, reproduction, and mammary cancer. He was also an excellent mentor to many researchers who, in turn, advanced these endeavors. He left an indelible mark on all of us that had the privilege of meeting him.

Disclosure Summary: Fred vom Saal worked as a consultant and provided expert testimony (<$10K). The authors have nothing to disclose.

## References


1. **National Toxicology Program** 2001 National Toxicology Program's report of the endocrine disruptors low dose peer review. Research Triangle Park, NC: National Institute of Environmental Health Sciences
2. **Melnick R, Lucier G, Wolfe M, Hall R, Stancel G, Prins G, Gallo M, Reuhl K, Ho SM, Brown T, Moore J, Leakey J, Haseman J, Kohn M** 2002 Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. Environ Health Perspect 110:427–431
3. **Welshons WV, Nagel SC, vom Saal FS** 2006 Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology 147:S56–S69
4. **Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV** 2007 Human exposure to bisphenol A (BPA). Reprod Toxicol 24:139–177
5. **Brucker-Davis F, Thayer K, Colborn T** 2001 Significant effects of mild endogenous hormonal changes in humans: considerations for low-dose testing. Environ Health Perspect 109:21–26
6. **Braun JM, Yolton K, Dietrich KN, Hornung R, Ye X, Calafat AM, Lanphear BP** 2009 Prenatal bisphenol A exposure and early childhood behavior. Environ Health Perspect 117:1945–1952
7. **Meeker JD, Barr DB, Hauser R** 2009 Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. Reprod Toxicol 27:155–160
8. **Weuve J, Hauser R, Calafat AM, Missmer SA, Wise LA** 2010 Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999–2004. Environ Health Perspect 118:825–832
9. **Meeker JD, Sathyanarayana S, Swan SH** 2009 Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos Trans R Soc Lond B Biol Sci 364:2097–2113
10. **Swan SH** 2008 Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res 108:177–184
11. **Akinbami LJ, Lynch CD, Parker JD, Woodruff TJ** 2010 The association between childhood asthma prevalence and monitored air pollutants in metropolitan areas, United States, 2001–2004. Environ Res 110:294–301
12. **Stillerman KP, Mattison DR, Giudice LC, Woodruff TJ** 2008 Environmental exposures and adverse pregnancy outcomes: a review of the science. Reprod Sci 15:631–650
13. **Grün F** 2010 Obesogens. Curr Opin Endocrinol Diabetes Obes 17:453–459
14. **Soto AM, Sonnenschein C** 2010 Environmental causes of cancer: endocrine disruptors as carcinogens. Nat Rev Endocrinol 6:363–370
15. **Meeker JD** 2010 Exposure to environmental endocrine disrupting compounds and men's health. Maturitas 66:236–241
16. **Hatch EE, Nelson JW, Stahlhut RW, Webster TF** 2010 Association of endocrine disruptors and obesity: perspectives from epidemiological studies. Int J Androl 33:324–332
17. **Hsu ST, Ma CI, Hsu SK, Wu SS, Hsu NH, Yeh CC, Wu SB** 1985 Discovery and epidemiology of PCB poisoning in Taiwan: a four-year followup. Environ Health Perspect 59:5–10
18. **Pesatori AC, Consonni D, Bachetti S, Zocchetti C, Bonzini M, Baccarelli A, Bertazzi PA** 2003 Short- and long-term morbidity and mortality in the population exposed to dioxin after the "Seveso accident". Ind Health 41:127–138
19. **Anderson HA, Wolff MS, Lilis R, Holstein EC, Valciukas JA, Anderson KE, Petrocci M, Sarkozi L, Selikoff IJ** 1979 Symptoms and clinical abnormalities following ingestion of polyborminated-biphenyl-contaminated food products. Ann NY Acad Sci 320:684–702
20. **Villeneuve S, Cyr D, Lynge E, Orsi L, Sabroe S, Merletti F, Gorini G, Morales-Suarez-Varela M, Ahrens W, Baumgardt-Elms C, Kaerlev L, Eriksson M, Hardell L, Févotte J, Guénel P** 2010 Occupation and occupational exposure to endocrine disrupting chemicals in male breast cancer: a case-control study in Europe. Occup Environ Med 67:837–844
21. **Li D, Zhou Z, Qing D, He Y, Wu T, Miao M, Wang J, Weng X, Ferber JR, Herrinton LJ, Zhu Q, Gao E, Checkoway H, Yuan W** 2010 Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. Hum Reprod 25:519–527
22. **Queiroz EK, Waissmann W** 2006 Occupational exposure and effects on the male reproductive system. Cad Saude Publica 22:485–493
23. **Centers for Disease Control** 2008 National Biomonitoring Program. Atlanta, GA: Centers for Disease Control, Prevention
24. **Kuklenyik Z, Ye X, Needham LL, Calafat AM** 2009 Automated solid-phase extraction approaches for large scale biomonitoring studies. J Chromatogr Sci 47:12–18
25. **Umweltbundesamt** 2009 Health and environmental hygiene: German environmental survey. Umweltbundesamt Dessau-Rosslau, Berlin, Germany
26. **Ha MH, Lee DH, Son HK, Park SK, Jacobs Jr DR** 2009 Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hyper-

tension: results from the National Health and Nutrition Examination Survey 1999–2002. J Hum Hypertens 23: 274–286

27. **vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, Farabollini F, Guillette Jr LJ, Hauser R, Heindel JJ, Ho SM, Hunt PA, Iguchi T, Jobling S, Kanno J, Keri RA, Knudsen KE, Laufer H, LeBlanc GA, Marcus M, McLachlan JA, Myers JP, Nadal A, Newbold RR, Olea N,** *et al.* 2007 Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol 24:131–138
28. **Crain DA, Eriksen M, Iguchi T, Jobling S, Laufer H, LeBlanc GA, Guillette Jr LJ** 2007 An ecological assessment of bisphenol-A: evidence from comparative biology. Reprod Toxicol 24:225–239
29. **Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, vom Saal FS** 2007 In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol 24:199–224
30. **Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM** 2007 In vitro molecular mechanisms of bisphenol A action. Reprod Toxicol 24:178–198
31. **Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM** 2009 Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocrine Reviews 30:75–95
32. **Keri RA, Ho SM, Hunt PA, Knudsen KE, Soto AM, Prins GS** 2007 An evaluation of evidence for the carcinogenic activity of bisphenol A. Reprod Toxicol 24:240–252
33. **U.S. Food and Drug Administration** 2008 Draft assessment of bisphenol A for use in food contact applications. Washington, DC: Department of Health and Human Services
34. **U.S. Food and Drug Administration** 2010 Update on bisphenol A (BPA) for use in food: January 2010. Washington, DC: Department of Health and Human Services
35. **Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO** 1995 The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 103(Suppl 7):113–122
36. **Nagel SC, vom Saal FS, Welshons WV** 1999 Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum. J Steroid Biochem Mol Biol 69:343–357
37. **Soto AM, Chung KL, Sonnenschein C** 1994 The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ Health Perspect 102:380–383
38. **Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS** 2003 Large effects from small exposures: I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect 111:994–1006
39. **Kochukov MY, Jeng YJ, Watson CS** 2009 Alkylphenol xenoestrogens with varying carbon chain lengths differentially and potently activate signaling and functional responses in GH3/B6/F10 somatomammotropes. Environ Health Perspect 117:723–730
40. **Alyea RA, Watson CS** 2009 Differential regulation of dopamine transporter function and location by low concentrations of environmental estrogens and 17$\beta$-estradiol. Environ Health Perspect 117:778–783
41. **Wozniak AL, Bulayeva NN, Watson CS** 2005 Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-$\alpha$ mediated $Ca^{2+}$ fluxes and prolactin release in GH3/B6 pituitary tumor cells. Environ Health Perspect 113:431–439
42. **Kohn MC, Melnick RL** 2002 Biochemical origins of the non-monotonic receptor-mediated dose-response. J Mol Endocrinol 29:113–123
43. **Conolly RB, Lutz WK** 2004 Nonmonotonic dose-response relationships: mechanistic basis, kinetic modeling, and implications for risk assessment. Toxicol Sci 77:151–157
44. **Zsarnovszky A, Le HH, Wang HS, Belcher SM** 2005 Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. Endocrinology 146:5388–5396
45. **Wong JK, Le HH, Zsarnovszky A, Belcher SM** 2003 Estrogens and ICI182,780 (Faslodex) modulate mitosis and cell death in immature cerebellar neurons via rapid activation of p44/p42 mitogen-activated protein kinase. J Neurosci 23:4984–4995
46. **Querfeld U, Mak RH** 2010 Vitamin D deficiency and toxicity in chronic kidney disease: in search of the therapeutic window. Pediatr Nephrol 25:2413–2430
47. **Cook R, Calabrese EJ** 2006 The importance of hormesis to public health. Environ Health Perspect 114:1631–1635
48. **Thayer KA, Melnick R, Huff J, Burns K, Davis D** 2006 Hormesis: a new religion? Environ Health Perspect 114: A632–A633
49. **Weltje L, vom Saal FS, Oehlmann J** 2005 Reproductive stimulation by low doses of xenoestrogens contrasts with the view of hormesis as an adaptive response. Hum Exp Toxicol 24:431–437
50. **Thayer KA, Melnick R, Burns K, Davis D, Huff J** 2005 Fundamental flaws of hormesis for public health decisions. Environ Health Perspect 113:1271–1276
51. **Beronius A, Rudén C, Håkansson H, Hanberg A** 2010 Risk to all or none? A comparative analysis of controversies in the health risk assessment of bisphenol A. Reprod Toxicol 29:132–146
52. **Bellinger DC** 2004 What is an adverse effect? A possible resolution of clinical and epidemiological perspectives on neurobehavioral toxicity. Environ Res 95:394–405
53. **Foster PM, McIntyre BS** 2002 Endocrine active agents: implications of adverse and non-adverse changes. Toxicol Pathol 30:59–65
54. **Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL** 2005 Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 113:1056–1061
55. **McEwen Jr GN, Renner G** 2006 Validity of anogenital distance as a marker of *in utero* phthalate exposure. Environ Health Perspect 114:A19–A20; author reply A20–A21
56. **Weiss B** 2006 Anogenital distance: defining "normal." Environ Health Perspect 114:A399; author reply A399

57. **Witorsch RJ** 2002 Low-dose *in utero* effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. Food Chem Toxicol 40:905–912
58. **O'Lone R, Frith MC, Karlsson EK, Hansen U** 2004 Genomic targets of nuclear estrogen receptors. Mol Endocrinol 18:1859–1875
59. **Schulkin J** 2011 Evolutionary conservation of glucocorticoids and corticotropin releasing hormone: behavioral and physiological adaptations. Brain Res 1392:27–46
60. **Williams GR, Franklyn JA** 1994 Physiology of the steroid-thyroid hormone nuclear receptor superfamily. Baillieres Clin Endocrinol Metab 8:241–266
61. **Enmark E, Gustafsson JA** 1999 Oestrogen receptors: an overview. J Intern Med 246:133–138
62. **U.S. Food and Drug Administration** 2009 Information for consumers (drugs). In: The beginnings: laboratory and animal studies. Washington, DC: Department of Health and Human Services
63. **Mittendorf R** 1995 Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) *in utero*. Teratology 51:435–445
64. **McLachlan JA** 2006 Commentary: prenatal exposure to diethylstilbestrol (DES): a continuing story. Int J Epidemiol 35:868–870
65. **Newbold RR, Jefferson WN, Padilla-Banks E** 2007 Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. Reprod Toxicol 24: 253–258
66. **Palmer JR, Wise LA, Hatch EE, Troisi R, Titus-Ernstoff L, Strohsnitter W, Kaufman R, Herbst AL, Noller KL, Hyer M, Hoover RN** 2006 Prenatal diethylstilbestrol exposure and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 15:1509–1514
67. **Soto AM, Vandenberg LN, Maffini MV, Sonnenschein C** 2008 Does breast cancer start in the womb? Basic Clin Pharmacol Toxicol 102:125–133
68. **Kamrin MA** 2007 The "low dose" hypothesis: validity and implications for human risk. Int J Toxicol 26:13–23
69. **Myers JP, vom Saal FS, Akingbemi BT, Arizono K, Belcher S, Colborn T, Chahoud I, Crain DA, Farabollini F, Guillette Jr LJ, Hassold T, Ho SM, Hunt PA, Iguchi T, Jobling S, Kanno J, Laufer H, Marcus M, McLachlan JA, Nadal A, Oehlmann J, Olea N, Palanza P, Parmigiani S, Rubin BS,** *et al.* 2009 Why public health agencies cannot depend upon 'Good Laboratory Practices' as a criterion for selecting data: the case of bisphenol-A. Environ Health Perspect 117: 309–315
70. **Myers JP, Zoeller RT, vom Saal FS** 2009 A clash of old and new scientific concepts in toxicity, with important implications for public health. Environ Health Perspect 117: 1652–1655
71. **vom Saal FS, Akingbemi BT, Belcher SM, Crain DA, Crews D, Guidice LC, Hunt PA, Leranth C, Myers JP, Nadal A, Olea N, Padmanabhan V, Rosenfeld CS, Schneyer A, Schoenfelder G, Sonnenschein C, Soto AM, Stahlhut RW, Swan SH, Vandenberg LN, Wang HS, Watson CS, Welshons WV, Zoeller RT** 2010 Flawed experimental design reveals the need for guidelines requiring appropriate positive controls in endocrine disruption research. Toxicol Sci 115:612–613; author reply 614–620
72. **vom Saal FS, Myers JP** 2010 Good laboratory practices are not synonymous with good scientific practices, accurate reporting, or valid data. Environ Health Perspect 118:A60
73. **Travis GD** 1981 Replicating replication? Aspects of the social construction of learning in planarian worms. Social Studies Sci 11:11–32
74. **Phillips CV, Goodman KJ** 2004 The missed lessons of Sir Austin Bradford Hill. Epidemiol Pespect Innov 1:3
75. **vom Saal FS, Hughes C** 2005 An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect 113: 926–933
76. **Hayes TB** 2004 There is no denying this: defusing the confusion about atrazine. BioScience 54:1138–1149
77. **vom Saal FS, Welshons WV** 2006 Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A. Environmental Research 100:50–76
78. **Bern HA, Edery M, Mills KT, Kohrman AF, Mori T, Larson L** 1987 Long-term alterations in histology and steroid receptor levels of the genital tract and mammary gland following neonatal exposure of female BALB/cCrgl mice to various doses of diethylstilbestrol. Cancer Res 47:4165–4172
79. **Krimsky S** 2003 Hormonal chaos: the scientific and social origins of the environmental endocrine hypothesis. Baltimore: Johns Hopkins University Press
80. **Barker DJ** 2007 The origins of the developmental origins theory. J Intern Med 261:412–417
81. **Barker DJP** 2004 The developmental origins of adult disease. J Am Coll Nutr 23:588S–595S
82. **Sharpe RM, Skakkebaek NE** 1993 Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341:1392–1395
83. **Trichopoulos D** 1990 Is breast cancer inititated *in utero*? Epidemiology 1:95–96
84. **Heindel JJ** 2006 Role of exposure to environmental chemicals in the developmental basis of reproductive disease and dysfunction. Semin Reprod Med 24:168–177
85. **Crain DA, Janssen SJ, Edwards TM, Heindel J, Ho SM, Hunt P, Iguchi T, Juul A, McLachlan JA, Schwartz J, Skakkebaek N, Soto AM, Swan S, Walker C, Woodruff TK, Woodruff TJ, Giudice LC, Guillette Jr LJ** 2008 Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. Fertil Steril 90: 911–940
86. **Heindel JJ** 2005 The fetal basis of adult disease: Role of environmental exposures: introduction. Birth Defects Res A Clin Mol Teratol 73:131–132
87. **Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G** 2010 Urine, serum and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect 118:1055–1070
88. **Hays SM, Aylward LL** 2009 Using biomonitoring equivalents to interpret human biomonitoring data in a public health risk context. J Appl Toxicol 29:275–288
89. **Clewell HJ, Tan YM, Campbell JL, Andersen ME** 2008 Quantitative interpretation of human biomonitoring data. Toxicol Appl Pharmacol 231:122–133
90. **Hayes TB, Case P, Chui S, Chung D, Haeffele C, Haston K, Lee M, Mai VP, Marjuoa Y, Parker J, Tsui M** 2006 Pesticide mixtures, endocrine disruption, and amphibian de-

clines: are we underestimating the impact? Environ Health Perspect 114:40–50
91. **Woodruff TJ, Zota AR, Schwartz JM** 2011 Environmental chemicals in pregnant women in the US: NHANES 2003–2004. Environ Health Perspect 119:878–885
92. **Young SS, Yu M** 2009 Association of bisphenol A with diabetes and other abnormalities. JAMA 301:720–721
93. **Smith GD, Ebrahim S** 2002 Data dredging, bias, or confounding. BMJ 325:1437–1438
94. **Marshall JR** 1990 Data dredging and noteworthiness. Epidemiology 1:5–7
95. **Vandenbroucke JP** 2008 Observational research, randomised trials, and two views of medical science. PLoS Medicine 5:e67
96. **Greenland S** 2007 Commentary: on 'quality in epidemiological research: should we be submitting papers before we have the results and submitting more hypothesis generating research?'. Int J Epidemiol 36:944–945
97. **Melzer D, Lang IA, Galloway TS** 2009 Reply to Young and Yu: association of bisphenol A with diabetes and other abnormalities. JAMA 301:721–722
98. **Wigle DT, Arbuckle TE, Turner MC, Bérubé A, Yang Q, Liu S, Krewski D** 2008 Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J Toxicol Environ Health B Crit Rev 11:373–517
99. **Watson CS, Gametchu B** 1999 Membrane-initiated steroid actions and the proteins that mediate them. Proc Soc Exp Biol Med 220:9–19
100. **Frühbeck G** 2006 Intracellular signalling pathways activated by leptin. Biochem J 393:7–20
101. **George JW, Dille EA, Heckert LL** 2011 Current concepts of follicle-stimulating hormone receptor gene regulation. Biol Reprod 84:7–17
102. **Cheng SY, Leonard JL, Davis PJ** 2010 Molecular aspects of thyroid hormone actions. Endocr Rev 31:139–170
103. **Kress E, Samarut J, Plateroti M** 2009 Thyroid hormones and the control of cell proliferation or cell differentiation: paradox or duality? Mol Cell Endocrinol 313:36–49
104. **Fu M, Wang C, Zhang X, Pestell RG** 2004 Acetylation of nuclear receptors in cellular growth and apoptosis. Biochem Pharmacol 68:1199–1208
105. **Katzenellenbogen BS, Montano MM, Ediger TR, Sun J, Ekena K, Lazennec G, Martini PG, McInerney EM, Delage-Mourroux R, Weis K, Katzenellenbogen JA** 2000 Estrogen receptors: selective ligands, partners, and distinctive pharmacology. Recent Prog Horm Res 55:163–193; discussion 194–195
106. **Zhao C, Dahlman-Wright K, Gustafsson JA** 2008 Estrogen receptor $\beta$: an overview and update. Nucl Recept Signal 6:e003
107. **Neill JD** 2005 Knobil and Neill's physiology of reproduction. 3rd ed. New York: Academic Press
108. **Jones KA** 1996 Summation of basic endocrine data. In: Gass GH, Kaplan HM, eds. Handbook of endocrinology. 2nd ed. New York: CRC Press; 1–42
109. **Stokes WS** 2004 Selecting appropriate animal models and experimental designs for endocrine disruptor research and testing studies. ILAR J 45:387–393
110. **May M, Moran JF, Kimelberg H, Triggle DJ** 1967 Studies on the noradrenaline $\alpha$-receptor. II. Analysis of the "spare-receptor" hypothesis and estimation of the concentration of $\alpha$-receptors in rabbit aorta. Mol Pharmacol 3:28–36
111. **Zhu BT** 1996 Rational design of receptor partial aganists and possible mechanisms of receptor partial activation: a theory. J Theor Biol 181:273–291
112. **Gan EH, Quinton R** 2010 Physiological significance of the rhythmic secretion of hypothalamic and pituitary hormones. Prog Brain Res 181:111–126
113. **Naftolin F, Garcia-Segura LM, Horvath TL, Zsarnovszky A, Demir N, Fadiel A, Leranth C, Vondracek-Klepper S, Lewis C, Chang A, Parducz A** 2007 Estrogen-induced hypothalamic synaptic plasticity and pituitary sensitization in the control of the estrogen-induced gonadotrophin surge. Reprod Sci 14:101–116
114. **Son GH, Chung S, Kim K** 2011 The adrenal peripheral clock: glucocorticoid and the circadian timing system. Front Neuroendocrinol 32:451–465
115. **Urbanski HF** 2011 Role of circadian neuroendocrine rhythms in the control of behavior and physiology. Neuroendocrinology 93:211–222
116. **National Research Council** 1999 Hormonally active agents in the environment. Washington, DC: National Academy Press
117. **Eick GN, Thornton JW** 2011 Evolution of steroid receptors from an estrogen-sensitive ancestral receptor. Mol Cell Endocrinol 334:31–38
118. **Sheehan DM** 2000 Activity of environmentally relevant low doses of endocrine disruptors and the bisphenol A controversy: initial results confirmed. Proc Soc Exp Biol Med 224:57–60
119. **Hayes TB, Anderson LL, Beasley VR, de Solla SR, Iguchi T, Ingraham H, Kestemont P, Kniewald J, Kniewald Z, Langlois VS, Luque EH, McCoy KA, Muñoz-de-Toro M, Oka T, Oliveira CA, Orton F, Ruby S, Suzawa M, Tavera-Mendoza LE, Trudeau VL, Victor-Costa AB, Willingham E** 2011 Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. J Steroid Biochem Mol Biol 127:64–73
120. **Beato M, Klug J** 2000 Steroid hormone receptors: an update. Hum Reprod Update 6:225–236
121. **Watson CS, Bulayeva NN, Wozniak AL, Finnerty CC** 2005 Signaling from the membrane via membrane estrogen receptor-$\alpha$: estrogens, xenoestrogens, and phytoestrogens. Steroids 70:364–371
122. **Powell CE, Soto AM, Sonnenschein C** 2001 Identification and characterization of membrane estrogen receptor from MCF7 estrogen-target cells. J Steroid Biochem Mol Biol 77:97–108
123. **Levin ER** 2011 Extranuclear steroid receptors: roles in modulation of cell functions. Mol Endocrinol 25:377–384
124. **Levin ER** 2009 Plasma membrane estrogen receptors. Trends Endocrinol Metab 20:477–482
125. **Thomas P, Dong J** 2006 Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. J Steroid Biochem Mol Biol 102:175–179
126. **Kenealy BP, Keen KL, Terasawa E** 2011 Rapid action of estradiol in primate GnRH neurons: The role of estrogen receptor $\alpha$ and estrogen receptor $\beta$. Steroids 76:861–866
127. **Watson CS, Bulayeva NN, Wozniak AL, Alyea RA** 2007

Xenoestrogens are potent activators of nongenomic estrogenic responses. Steroids 72:124–134
128. **Ropero AB, Alonso-Magdalena P, Ripoll C, Fuentes E, Nadal A** 2006 Rapid endocrine disruption: environmental estrogen actions triggered outside the nucleus. J Steroid Biochem Mol Biol 102:163–169
129. **Nadal A, Alonso-Magdalena P, Ripoll C, Fuentes E** 2005 Disentangling the molecular mechanisms of action of endogenous and environmental estrogens. Pflugers Arch 449: 335–343
130. **Thomas P, Pang Y, Filardo EJ, Dong J** 2005 Identity of an estogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology 146:624–632
131. **Nadal A, Ropero AB, Laribi O, Maillet M, Fuentes E, Soria B** 2000 Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor $\alpha$ and estrogen receptor $\beta$. Proc Natl Acad Sci USA 97:11603–11608
132. **Tanabe N, Kimoto T, Kawato S** 2006 Rapid $Ca^{2+}$ signaling induced by bisphenol A in cultured rat hippocampal neurons. Neuro Endocrinol Lett 27:97–104
133. **Ruehlmann DO, Steinert JR, Valverde MA, Jacob R, Mann GE** 1998 Environmental estrogenic pollutants induce acute vascular relaxation by inhibiting L-type $Ca^{2+}$ channels in smooth muscle cells. FASEB J 12:613–619
134. **Walsh DE, Dockery P, Doolan CM** 2005 Estrogen receptor independent rapid non-genomic effects of environmental estrogens on $[Ca^{2+}]$ in human breast cancer cells. Mol Cell Endocrinol 230:23–30
135. **Shioda T, Chesnes J, Coser KR, Zou L, Hur J, Dean KL, Sonnenschein C, Soto AM, Isselbacher KJ** 2006 Importance of dosage standardization for interpreting transcriptomal signature profiles: evidence from studies of xenoestrogens. Proc Natl Acad Sci USA 103:12033–12038
136. **Ryan BC, Vandenbergh JG** 2002 Intrauterine position effects. Neurosci Biobehav Rev 26:665–678
137. **Muñoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM** 2005 Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. Endocrinology 146:4138–4147
138. **Wadia PR, Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM** 2007 Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains. Environ Health Perspect 115:592–598
139. **Prins GS, Birch L, Tang WY, Ho SM** 2007 Developmental estrogen exposures predispose to prostate carcinogenesis with aging. Reprod Toxicol 23:374–382
140. **Prins GS, Tang WY, Belmonte J, Ho SM** 2008 Perinatal exposure to oestradiol and bisphenol A alters the prostate epigenome and increases susceptibility to carcinogenesis. Basic Clin Pharmacol Toxicol 102:134–138
141. **Prins GS, Ye SH, Birch L, Ho SM, Kannan K** 2011 Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats. Reprod Toxicol 31:1–9
142. **Bjørnerem A, Straume B, Midtby M, Fønnebø V, Sundsfjord J, Svartberg J, Acharya G, Oian P, Berntsen GK** 2004 Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: the Tromso Study. J Clin Endocrinol Metab 89:6039–6047
143. **Silva E, Rajapakse N, Kortenkamp A** 2002 Something from "nothing": eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ Sci Technol 36:1751–1756
144. **Soto AM, Fernandez MF, Luizzi MF, Oles Karasko AS, Sonnenschein C** 1997 Developing a marker of exposure to xenoestrogen mixtures in human serum. Environ Health Perspect 105:647–654
145. **Crofton KM** 2008 Thyroid disrupting chemicals: mechanisms and mixtures. Int J Androl 31:209–223
146. **Montano MM, Welshons WV, vom Saal FS** 1995 Free estradiol in serum and brain uptake of estradiol during fetal and neonatal sexual differentiation in female rats. Biol Reprod 53:1198–1207
147. **Nunez EA, Benassayag C, Savu L, Vallette G, Delorme J** 1979 Oestrogen binding function of $\alpha$ 1-fetoprotein. J Steroid Biochem 11:237–243
148. **Milligan SR, Khan O, Nash M** 1998 Competitive binding of xenobiotic oestrogens to rat $\alpha$-fetoprotein and to sex steroid binding proteins in human and rainbow trout (*Oncorhynchus mykiss*) plasma. Gen Comp Endocrinol 112: 89–95
149. **Sheehan DM, Young M** 1979 Diethylstilbestrol and estradiol binding to serum albumin and pregnancy plasma of rat and human. Endocrinology 104:1442–1446
150. **Déchaud H, Ravard C, Claustrat F, de la Perrière AB, Pugeat M** 1999 Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). Steroids 64: 328–334
151. **Liu SV, Schally AV, Hawes D, Xiong S, Fazli L, Gleave M, Cai J, Groshen S, Brands F, Engel J, Pinski J** 2010 Expression of receptors for luteinizing hormone-releasing hormone (LH-RH) in prostate cancers following therapy with LH-RH agonists. Clin Cancer Res 16:4675–4680
152. **Piccart M, Parker LM, Pritchard KI** 2003 Oestrogen receptor downregulation: an opportunity for extending the window of endocrine therapy in advanced breast cancer. Ann Oncol 14:1017–1025
153. **Grandien K, Berkenstam A, Gustafsson JA** 1997 The estrogen receptor gene: promoter organization and expression. Int J Biochem Cell Biol 29:1343–1369
154. **Morani A, Warner M, Gustafsson JA** 2008 Biological functions and clinical implications of oestrogen receptors alfa and $\beta$ in epithelial tissues. J Intern Med 264:128–142
155. **Mostaghel EA, Montgomery RB, Lin DW** 2007 The basic biochemistry and molecular events of hormone therapy. Curr Urol Rep 8:224–232
156. **Phoenix CH, Goy RW, Gerall AA, Young WC** 1959 Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 65:369–382
157. **Vom Saal FS, Moyer CL** 1985 Prenatal effects on reproductive capacity during aging in female mice. Biol Reprod 32:1116–1126
158. **Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, Nadal A** 2010 Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ Health Perspect 118:1243–1250
159. **Even MD, Dhar MG, vom Saal FS** 1992 Transport of steroids between fetuses via amniotic fluid in relation to the

intrauterine position phenomenon in rats. J Reprod Fertil 96:709–716
160. **vom Saal FS, Quadagno DM, Even MD, Keisler LW, Keisler DH, Khan S** 1990 Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. Biol Reprod 43:751–761
161. **vom Saal FS, Bronson FH** 1978 *In utero* proximity of female mouse fetuses to males: effect on reproductive performance during later life. Biol Reprod 19:842–853
162. **Kinsley CH, Konen CM, Miele JL, Ghiraldi L, Svare B** 1986 Intrauterine position modulates maternal behaviors in female mice. Physiol Behav 36:793–799
163. **Gandelman R, vom Saal FS, Reinisch JM** 1977 Contiguity to male foetuses affects morphology and behaviour of female mice. Nature 266:722–724
164. **Palanza P, Parmigiani S, vom Saal FS** 1995 Urine marking and maternal aggression of wild female mice in relation to anogenital distance at birth. Physiol Behav 58:827–835
165. **vom Saal FS, Grant WM, McMullen CW, Laves KS** 1983 High fetal estrogen concentrations: correlation with increased adult sexual activity and decreased aggression in male mice. Science 220:1306–1309
166. **Palanza P, Morley-Fletcher S, Laviola G** 2001 Novelty seeking in periadolescent mice: sex differences and influence of intrauterine position. Physiol Behav 72:255–262
167. **Clark MM, vom Saal FS, Galef Jr BG** 1992 Intrauterine positions and testosterone levels of adult male gerbils are correlated. Physiol Behav 51:957–960
168. **vom Saal FS** 1989 Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses *in utero*. J Anim Sci 67:1824–1840
169. **vom Saal FS** 1989 The production of and sensitivity to cues that delay puberty and prolong subsequent oestrous cycles in female mice are influenced by prior intrauterine position. J Reprod Fertil 86:457–471
170. **Vom Saal FS, Even MD, Quadagno DM** 1991 Effects of maternal stress on puberty, fertility and aggressive behavior of female mice from different intrauterine positions. Physiol Behav 49:1073–1078
171. **vom Saal FS, Pryor S, Bronson FH** 1981 Effects of prior intrauterine position and housing on oestrous cycle length in adolescent mice. Journal of Reproduction, Fertility 62: 33–37
172. **Vandenbergh JG, Huggett CL** 1994 Mother's prior intrauterine position affects the sex ratio of her offspring in house mice. Proc Natl Acad Sci USA 91:11055–11059
173. **Vandenbergh JG, Huggett CL** 1995 The anogenital distance index, a predictor of the intrauterine position effects on reproduction in female house mice. Lab Anim Sci 45: 567–573
174. **Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS** 1999 Exposure to bisphenol A advances puberty. Nature 401:763–764
175. **vom Saal FS, Bronson FH** 1980 Variation in length of estrous cycles in mice due to former intrauterine proximity to male fetuses. Biol Reprod 22:777–780
176. **Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, Rubin BS, Soto AM** 2007 Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. Endocrinology 148:116–127
177. **Timms BG, Petersen SL, vom Saal FS** 1999 Prostate gland growth during development is stimulated in both male and female rat fetuses by intrauterine proximity to female fetuses. J Urol 161:1694–1701
178. **Nonneman DJ, Ganjam VK, Welshons WV, Vom Saal FS** 1992 Intrauterine position effects on steroid metabolism and steroid receptors of reproductive organs in male mice. Biol Reprod 47:723–729
179. **Clark MM, Bishop AM, vom Saal FS, Galef Jr BG** 1993 Responsiveness to testosterone of male gerbils from known intrauterine positions. Physiol Behav 53:1183–1187
180. **vom Saal FS, Bronson FH** 1980 Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. Science 208: 597–599
181. **Timms BG, Peterson RE, vom Saal FS** 2002 2,3,7,8-tetrachlorodibenzo-*p*-dioxin interacts with endogenous estradiol to disrupt prostate gland morphogenesis in male rat fetuses. Toxicol Sci 67:264–274
182. **Vandenbergh JG** 2004 Animal models and studies of *in utero* endocrine disruptor effects. ILAR J 45:438–442
183. **Clark MM, Crews D, Galef Jr BG** 1991 Concentrations of sex steroid hormones in pregnant and fetal Mongolian gerbils. Physiol Behav 49:239–243
184. **Satoh S, Hirata T, Miyake Y, Kaneda Y** 1997 The possibility of early estimation for fertility in bovine heterosexual twin females. J Vet Med Sci 59:221–222
185. **Padula AM** 2005 The freemartin syndrome: an update. Anim Reprod Sci 87:93–109
186. **Resnick SM, Gottesman II, McGue M** 1993 Sensation seeking in opposite-sex twins: an effect of prenatal hormones? Behav Genet 23:323–329
187. **McFadden D** 1993 A masculinizing effect on the auditory systems of human females having male co-twins. Proc Natl Acad Sci USA 90:11900–11904
188. **Cohen-Bendahan CC, Buitelaar JK, van Goozen SH, Cohen-Kettenis PT** 2004 Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls. Psychoneuroendocrinology 29:911–916
189. **Peper JS, Brouwer RM, van Baal GC, Schnack HG, van Leeuwen M, Boomsma DI, Kahn RS, Hulshoff Pol HE** 2009 Does having a twin brother make for a bigger brain? Eur J Endocrinol 160:739–746
190. **Cohen-Bendahan CC, Buitelaar JK, van Goozen SH, Orlebeke JF, Cohen-Kettenis PT** 2005 Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls. Horm Behav 47:230–237
191. **Loehlin JC, Martin NG** 2000 Dimensions of psychological masculinity-femininity in adult twins from opposite-sex and same-sex pairs. Behav Genet 30:19–28
192. **Rose RJ, Kaprio J, Winter T, Dick DM, Viken RJ, Pulkkinen L, Koskenvuo M** 2002 Femininity and fertility in sisters with twin brothers: prenatal androgenization? Cross-sex socialization? Psychol Sci 13:263–267
193. **Vuoksimaa E, Eriksson CJ, Pulkkinen L, Rose RJ, Kaprio J** 2010 Decreased prevalence of left-handedness among females with male co-twins: evidence suggesting prenatal tes-

tosterone transfer in humans? Psychoneuroendocrinology 35:1462–1472
194. **Elkadi S, Nicholls ME, Clode D** 1999 Handedness in opposite and same-sex dizygotic twins: testing the testosterone hypothesis. Neuroreport 10:333–336
195. **Lummaa V, Pettay JE, Russell AF** 2007 Male twins reduce fitness of female co-twins in humans. Proc Natl Acad Sci USA 104:10915–10920
196. **van Anders SM, Vernon PA, Wilbur CJ** 2006 Finger-length ratios show evidence of prenatal hormone-transfer between opposite-sex twins. Horm Behav 49:315–319
197. **Culbert KM, Breedlove SM, Burt SA, Klump KL** 2008 Prenatal hormone exposure and risk for eating disorders. Arch Gen Psychiatry 65:329–336
198. **Glinianaia SV, Magnus P, Harris JR, Tambs K** 1998 Is there a consequence for fetal growth of having an unlike-sexed cohabitant *in utero*? Int J Epidemiol 27:657–659
199. **Cerhan JR, Kushi LH, Olson JE, Rich SS, Zheng W, Folsom AR, Sellers TA** 2000 Twinship and risk of postmenopausal breast cancer. J Natl Cancer Inst 92:261–265
200. **Swerdlow AJ, De Stavola BL, Swanwick MA, Maconochie NES** 1997 Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. Lancet 350:1723–1728
201. **van de Beek C, Thijssen JH, Cohen-Kettenis PT, van Goozen SH, Buitelaar JK** 2004 Relationships between sex hormones assessed in amniotic fluid, and maternal and umbilical cord serum: what is the best source of information to investigate the effects of fetal hormone exposure? Horm Behav 46:663–669
202. **Sakai LM, Baker LA, Jacklin CN, Shulman I** 1991 Sex steroids at birth: genetic and environmental variation and covariation. Dev Psychobiol 24:559–570
203. **Cohen-Bendahan CC, van Goozen SH, Buitelaar JK, Cohen-Kettenis PT** 2005 Maternal serum steroid levels are unrelated to fetal sex: a study in twin pregnancies. Twin Res Hum Genet 8:173–177
204. **Johnson MR, Abbas A, Nicolaides KH** 1994 Maternal plasma levels of human chorionic gonadotropin, oestradiol and progesterone in multifetal pregnancies before and after fetal reduction. J Endocrinol 143:309–312
205. **Vom Saal FS, Richter CA, Ruhlen RR, Nagel SC, Timms BG, Welshons WV** 2005 The importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose studies of bisphenol A. Birth Defects Res A Clin Mol Teratol 73:140–145
206. **Spearow JL, Doemeny P, Sera R, Leffler R, Barkley M** 1999 Genetic variation in susceptibility to endocrine disruption by estrogen in mice. Science 285:1259–1261
207. **Spearow JL, O'Henley P, Doemeny P, Sera R, Leffler R, Sofos T, Barkley M** 2001 Genetic variation in physiological sensitivity to estrogen in mice. APMIS 109:356–364
208. **Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS** 2005 Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. Proc Natl Acad Sci USA 102:7014–7019
209. **Cederroth CR, Nef S** 2009 Fetal programming of adult glucose homeostasis in mice. PLoS ONE 4:e7281
210. **Marty MS, Carney EW, Rowlands JC** 2011 Endocrine disruption: historical perspectives and its impact on the future of toxicology testing. Toxicol Sci 120:S93–S108
211. **Bonefeld-Jorgensen EC, Long M, Hofmeister MV, Vinggaard AM** 2007 Endocrine-disrupting potential of bisphenol A, bisphenol A dimethacrylate, 4-n-nonylphenol, and 4-n-octylphenol in vitro: new data and a brief review. Environ Health Perspect 115(Suppl 1):69–76
212. **Krüger T, Long M, Bonefeld-Jørgensen EC** 2008 Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. Toxicology 246:112–123
213. **Watson CS, Jeng YJ, Kochukov MY** 2010 Nongenomic signaling pathways of estrogen toxicity. Toxicol Sci 115: 1–11
214. **Weed DL** 2005 Weight of evidence: a review of concepts and methods. Risk Anal 25:1545–1557
215. **Linkov I, Loney D, Cormier S, Satterstrom FK, Bridges T** 2009 Weight-of-evidence evaluation in environmental assessment: review of qualitative and quantitative approaches. Sci Total Environ 407:5199–5205
216. **Schreider J, Barrow C, Birchfield N, Dearfield K, Devlin D, Henry S, Kramer M, Schappelle S, Solomon K, Weed DL, Embry MR** 2010 Enhancing the credibility of decisions based on scientific conclusions: transparency is imperative. Toxicol Sci 116:5–7
217. **Basketter D, Ball N, Cagen S, Carrillo JC, Certa H, Eigler D, Garcia C, Esch H, Graham C, Haux C, Kreiling R, Mehling A** 2009 Application of a weight of evidence approach to assessing discordant sensitisation datasets: implications for REACH. Regul Toxicol Pharmacol 55: 90–96
218. **Wright-Walters M, Volz C, Talbott E, Davis D** 2011 An updated weight of evidence approach to the aquatic hazard assessment of bisphenol A and the derivation a new predicted no effect concentration (Pnec) using a non-parametric methodology. Sci Total Environ 409:676–685
219. **Cooper RL, Kavlock RJ** 1997 Endocrine disruptors and reproductive development: a weight-of-evidence overview. J Endocrinol 152:159–166
220. **Popp JA, Crouch E, McConnell EE** 2006 A weight-of-evidence analysis of the cancer dose-response characteristics of 2,3,7,8-tetrachlorodibenzodioxin (TCDD). Toxicol Sci 89:361–369
221. **Goodman M, Squibb K, Youngstrom E, Anthony LG, Kenworthy L, Lipkin PH, Mattison DR, Lakind JS** 2010 Using systematic reviews and meta-analyses to support regulatory decision making for neurotoxicants: lessons learned from a case study of PCBs. Environ Health Perspect 118: 727–734
222. **Goodman JE, Witorsch RJ, McConnell EE, Sipes IG, Slayton TM, Yu CJ, Franz AM, Rhomberg LR** 2009 Weight-of-evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. Crit Rev Toxicol 39: 1–75
223. **Heindel JJ, vom Saal FS** 2008 Meeting report: batch-to-batch variability in estrogenic activity in commercial animal diets- importance and approaches for laboratory animal research. Environ Health Perspect 116:389–393
224. **Ruhlen RL, Taylor JA, Mao J, Kirkpatrick J, Welshons WV, vom Saal FS** 2011 Choice of animal feed can alter fetal steroid levels and mask developmental effects of endocrine disrupting chemicals. J Dev Origins Health Dis 2:36–48

225. **vom Saal FS, Richter CA, Mao J, Welshons WV** 2005 Commercial animal feed: variability in estrogenic activity and effects on body weight in mice. Birth Defects Res (Part A) 73:474–475
226. **Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, Ruhlen RL, Vom Saal FS, Welshons WV** 2003 Bisphenol A is released from polycarbonate animal cages into water at room temperature. Environ Health Perspect 111:1180–1187
227. **Koehler KE, Voigt RC, Thomas S, Lamb B, Urban C, Hassold T, Hunt PA** 2003 When disaster strikes: rethinking caging materials. Lab Anim (NY) 32:24–27
228. **Muhlhauser A, Susiarjo M, Rubio C, Griswold J, Gorence G, Hassold T, Hunt PA** 2009 Bisphenol A effects on the growing mouse oocyte are influenced by diet. Biol Reprod 80:1066–1071
229. **Tyl RW, Myers CB, Marr MC, Castillo NP, Veselica MM, Joiner RL, Dimond SS, Van Miller JP, Stropp GD, Waechter Jr JM, Hentges SG** 2008 One-generation reproductive toxicity study of dietary 17$\beta$-estradiol (E2; CAS no. 50-28-2) in CD-1 (Swiss) mice. Reprod Toxicol 25:144–160
230. **Ryan BC, Hotchkiss AK, Crofton KM, Gray Jr LE** 2010 *In utero* and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. Toxicol Sci 114:133–148
231. **Marty MS, Allen B, Chapin RE, Cooper R, Daston GP, Flaws JA, Foster PM, Makris SL, Mylchreest E, Sandler D, Tyl RW** 2009 Inter-laboratory control data for reproductive endpoints required in the OPPTS 870.3800/OECD 416 reproduction and fertility test. Birth Defects Res B Dev Reprod Toxicol 86:470–489
232. **Teng CT, Beard C, Gladwell W** 2002 Differential expression and estrogen response of lactoferrin gene in the female reproductive tract of mouse, rat, and hamster. Biol Reprod 67:1439–1449
233. **Aupperlee MD, Drolet AA, Durairaj S, Wang W, Schwartz RC, Haslam SZ** 2009 Strain-specific differences in the mechanisms of progesterone regulation of murine mammary gland development. Endocrinology 150:1485–1494
234. **Pepling ME, Sundman EA, Patterson NL, Gephardt GW, Medico L Jr, Wilson KI** 2010 Differences in oocyte development and estradiol sensitivity among mouse strains. Reproduction 139:349–357
235. **Wiklund JA, Gorski J** 1982 Genetic differences in estrogen-induced DNA synthesis in the rat pituitary: correlations with pituitary tumor susceptibility. Endocrinology 111: 1140–1149
236. **Wiklund J, Wertz N, Gorski J** 1981 A comparison of estrogen effects on uterine and pituitary growth and prolactin synthesis in F344 and Holtzman rats. Endocrinology 109:1700–1707
237. **Diel P, Schmidt S, Vollmer G, Janning P, Upmeier A, Michna H, Bolt HM, Degen GH** 2004 Comparative responses of three rat strains (DA/Han, Sprague-Dawley and Wistar) to treatment with environmental estrogens. Arch Toxicol 78:183–193
238. **Brossia LJ, Roberts CS, Lopez JT, Bigsby RM, Dynlacht JR** 2009 Interstrain differences in the development of pyometra after estrogen treatment of rats. J Am Assoc Lab Anim Sci 48:517–520
239. **Geis RB, Diel P, Degen GH, Vollmer G** 2005 Effects of genistein on the expression of hepatic genes in two rat strains (Sprague-Dawley and Wistar). Toxicol Lett 157: 21–29
240. **Roper RJ, Griffith JS, Lyttle CR, Doerge RW, McNabb AW, Broadbent RE, Teuscher C** 1999 Interacting quantitative trait loci control phenotypic variation in murine estradiol-regulated responses. Endocrinology 140:556–561
241. **Taylor JA, Welshons WV, Vom Saal FS** 2008 No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24h after administration in neonatal female mice. Reprod Toxicol 25:169–176
242. **European Food Safety Authority** 2007 Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2,2-biS(4-hydroxyphenyl)propane. EFSA J 428:1–75
243. **Vandenberg LN, Chahoud I, Padmanabhan V, Paumgartten FJ, Schoenfelder G** 2010 Biomonitoring studies should be used by regulatory agencies to assess human exposure levels and safety of bisphenol A. Environ Health Perspect 118:1051–1054
244. **Vandenberg LN** 2011 Exposure to bisphenol A in Canada: invoking the precautionary principle. CMAJ 183:1265–1270
245. **Stahlhut RW, Welshons WV, Swan SH** 2009 Bisphenol A data in NHANES suggest longer than expected half-life, substantial non-food exposure, or both. Environ Health Perspect 117:784–789
246. **Geens T, Goeyens L, Covaci A** 2011 Are potential sources for human exposure to bisphenol-A overlooked? Int J Hyg Environ Health 214:339–347
247. **Biedermann S, Tschudin P, Grob K** 2010 Transfer of bisphenol A from thermal printer paper to the skin. Anal Bioanal Chem 398:571–576
248. **Zalko D, Jacques C, Duplan H, Bruel S, Perdu E** 2011 Viable skin efficiently absorbs and metabolizes bisphenol A. Chemosphere 82:424–430
249. **Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, Hataya Y, Shimatsu A, Kuzuya H, Nakao K** 2002 Thyroid hormone action is disrupted by bisphenol A as an antagonist. J Clin Endocrinol Metab 87:5185–5190
250. **Zoeller RT, Bansal R, Parris C** 2005 Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. Endocrinology 146:607–612
251. **Lee HJ, Chattopadhyay S, Gong EY, Ahn RS, Lee K** 2003 Antiandrogenic effects of bisphenol A and nonphenol on the function of androgen receptor. Toxicol Sci 75:40–46
252. **Kwintkiewicz J, Nishi Y, Yanase T, Giudice LC** 2010 Peroxisome proliferator-activated receptor-$\gamma$ mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells. Environ Health Perspect 118:400–406
253. **Taylor JA, Vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain PL, Laffont CM, VandeVoort CA** 2011 Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. Environ Health Perspect 119:422–430
254. **Owens JW, Chaney JG** 2005 Weighing the results of differing 'low dose' studies of the mouse prostate by

Nagel, Cagen, and Ashby: quantification of experimental power and statistical results. Regul Toxicol Pharmacol 43:194–202
255. **Ashby J, Tinwell H, Odum J, Lefevre P** 2004 Natural variability and the influence of concurrent control values on the detection and interpretation of low-dose or weak endocrine toxicities. Environ Health Perspect 112:847–853
256. **Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV** 1997 Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ Health Perspect 105:70–76
257. **Gupta C** 2000 Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. Proc Soc Exp Biol Med 224:61–68
258. **Elswick BA, Welsch F, Janszen DB** 2000 Effect of different sampling designs on outcome of endocrine disruptor studies. Reprod Toxicol 14:359–367
259. **Chitra KC, Latchoumycandane C, Mathur PP** 2003 Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology 185:119–127
260. **Ramos JG, Varayoud J, Sonnenschein C, Soto AM, Muñoz De Toro M, Luque EH** 2001 Prenatal exposure to low doses of bisphenol A alters the periductal stroma and glandular cell function in the rat ventral prostate. Biol Reprod 65:1271–1277
261. **Ramos JG, Varayoud J, Kass L, Rodríguez H, Costabel L, Muñoz-De-Toro M, Luque EH** 2003 Bisphenol A induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats. Endocrinology 144:3206–3215
262. **Ogura Y, Ishii K, Kanda H, Kanai M, Arima K, Wang Y, Sugimura Y** 2007 Bisphenol A induces permanent squamous change in mouse prostatic epithelium. Differentiation 75:745–756
263. **Ho SM, Tang WY, Belmonte de Frausto J, Prins GS** 2006 Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 66:5624–5632
264. **Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, Inaguma S, Suzuki S, Shirai T** 2003 Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. J Toxicol Sci 28:165–171
265. **Ashby J, Tinwell H, Haseman J** 1999 Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed *in utero*. Regul Toxicol Pharmacol 30:156–166
266. **Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR** 1999 Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. Toxicol Sci 50:36–44
267. **Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR** 1999 Normal reproductive organ development in Wistar rats exposed to bisphenol A in the drinking water. Regul Toxicol Pharmacol 30:130–139
268. **Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A** 2001 Rat two-generation reproductive toxicity study of bisphenol A. Reprod Toxicol 15:505–523
269. **Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J** 2002 Normal sexual development of two strains of rat exposed *in utero* to low doses of bisphenol A. Toxicol Sci 68:339–348
270. **Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM** 2002 Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol Sci 68:121–146
271. **Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter Jr JM** 2008 Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicol Sci 104:362–384
272. **Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray Jr LE** 2008 Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat. Toxicol Sci 102:371–382
273. **Chapin RE, Adams J, Boekelheide K, Gray LE Jr, Hayward SW, Lees PS, McIntyre BS, Portier KM, Schnorr TM, Selevan SG, Vandenbergh JG, Woskie SR** 2008 NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res B Dev Reprod Toxicol 83:157–395
274. **Hennighausen L, Robinson GW** 1998 Think globally, act locally: the making of a mouse mammary gland. Genes Dev 12:449–455
275. **Lemmen JG, Broekhof JL, Kuiper GG, Gustafsson JA, van der Saag PT, van der Burg B** 1999 Expression of estrogen receptor $\alpha$ and $\beta$ during mouse embryogensis. Mech Dev 81:163–167
276. **Padilla-Banks E, Jefferson WN, Newbold RR** 2006 Neonatal exposure to the phytoestrogen genistein alters mammary gland growth and developmental programming of hormone receptor levels. Endocrinology 147:4871–4882
277. **Colerangle JB, Roy D** 1997 Profound effects of the weak environmental estrogen-like chemical bisphenol A on the growth of the mammary gland of Noble rats. J Steroid Biochem Mol Biol 60:153–160
278. **Bern HA, Mills KT, Jones LA** 1983 Critical period of neonatal estrogen exposure in occurence of mammary gland abnormalities in adult mice. Proc Soc Exp Biol Med 172: 239–242
279. **Markey CM, Coombs MA, Sonnenschein C, Soto AM** 2003 Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. Evol Dev 5:67–75
280. **Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM** 2001 *In utero* exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. Biol Reprod 65:1215–1223
281. **Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, Sonnenschein C, Rubin BS, Soto AM** 2008 Perinatal exposure to the xenoestrogen bisphenol-A induces mammary

intraductal hyperplasias in adult CD-1 mice. Reprod Toxicol 26:210–219
282. **Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J, Russo J** 2008 Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. J Endocrinol 196:101–112
283. **Ayyanan A, Laribi O, Schuepbach-Mallepell S, Schrick C, Gutierrez M, Tanos T, Lefebvre G, Rougemont J, Yalcin-Ozuysal O, Brisken C** 2011 Perinatal exposure to bisphenol A increases adult mammary gland progesterone response and cell number. Mol Endocrinol 25:1915–1923
284. **Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A** 2004 Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. Reprod Toxicol 18:803–811
285. **Jones LP, Sampson A, Kang HJ, Kim HJ, Yi YW, Kwon SY, Babus JK, Wang A, Bae I** 2010 Loss of BRCA1 leads to an increased sensitivity to bisphenol A. Toxicol Lett 199:261–268
286. **Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM** 2007 Induction of mammary gland ductal hyperplasias and carcinomas in situ following fetal bisphenol A exposure. Reprod Toxicol 23:383–390
287. **Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH, Muñoz-de-Toro M** 2007 Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. Environ Health Perspect 115:80–86
288. **Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J, Lamartiniere CA** 2009 Oral exposure to bisphenol A increases dimethylbenzanthracene-induced mammary cancer in rats. Environ Health Perspect 117:910–915
289. **Betancourt AM, Eltoum IA, Desmond RA, Russo J, Lamartiniere CA** 2010 *In utero* exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat. Environ Health Perspect 118:1614–1619
290. **Weber Lozada K, Keri RA** 2011 Bisphenol A increases mammary cancer risk in two distinct mouse models of breast cancer. Biol Reprod 85:490–497
291. **Betancourt AM, Mobley JA, Russo J, Lamartiniere CA** 2010 Proteomic analysis in mammary glands of rat offspring exposed *in utero* to bisphenol A. J Proteomics 73:1241–1253
292. **Lamartiniere CA, Jenkins S, Betancourt AM, Wang J, Russo J** 2011 Exposure to the endocrine disruptor bisphenol A alters susceptibility for mammary cancer. Horm Mol Biol Clin Investig 5:45–52
293. **Jenkins S, Wang J, Eltoum I, Desmond R, Lamartiniere CA** 2011 Chronic oral exposure to bisphenol A results in a non-monotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice. Environ Health Perspect 119:1604–1609
294. **Nikaido Y, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A** 2005 Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. In Vivo 19:487–494
295. **Yin H, Ito A, Bhattacharjee D, Hoshi M** 2006 A comparative study on the protective effects of 17$\beta$-estradiol, biochanin A and bisphenol A on mammary gland differentiation and tumorigenesis in rats. Indian J Exp Biol 44:540–546
296. **Yang M, Ryu JH, Jeon R, Kang D, Yoo KY** 2009 Effects of bisphenol A on breast cancer and its risk factors. Arch Toxicol 83:281–285
297. **Kortenkamp A** 2006 Breast cancer, oestrogens and environmental pollutants: a re-evaluation from a mixture perspective. Int J Androl 29:193–198
298. **Hunt PA, Susiarjo M, Rubio C, Hassold TJ** 2009 The bisphenol A experience: a primer for the analysis of environmental effects on mammalian reproduction. Biol Reprod 81:807–813
299. **Carr R, Bertasi F, Betancourt A, Bowers S, Gandy BS, Ryan P, Willard S** 2003 Effect of neonatal rat bisphenol a exposure on performance in the Morris water maze. J Toxicol Environ Health A 66:2077–2088
300. **Farabollini F, Porrini S, Dessì-Fulgherit F** 1999 Perinatal exposure to the estrogenic pollutant bisphenol A affects behavior in male and female rats. Pharmacol Biochem Behav 64:687–694
301. **Fujimoto T, Kubo K, Aou S** 2006 Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. Brain Res 1068:49–55
302. **Funabashi T, Kawaguchi M, Furuta M, Fukushima A, Kimura F** 2004 Exposure to bisphenol A during gestation and lactation causes loss of sex difference in corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of rats. Psychoneuroendocrinology 29:475–485
303. **Kubo K, Arai O, Omura M, Watanabe R, Ogata R, Aou S** 2003 Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. Neurosci Res 45:345–356
304. **Kubo K, Arai O, Ogata R, Omura M, Hori T, Aou S** 2001 Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behaviour in the rat. Neurosci Lett 304:73–76
305. **Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM** 2006 Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. Endocrinology 147:3681–3691
306. **Patisaul HB, Fortino AE, Polston EK** 2006 Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. Neurotoxicol Teratol 28:111–118
307. **Adewale HB, Todd KL, Mickens JA, Patisaul HB** 2011 The impact of neonatal bisphenol: a exposure on sexually dimorphic hypothalamic nuclei in the female rat. Neurotoxicology 32:38–49
308. **Wolstenholme JT, Rissman EF, Connelly JJ** 2011 The role of bisphenol A in shaping the brain, epigenome and behavior. Horm Behav 59:296–305
309. **Maffini MV, Rubin BS, Sonnenschein C, Soto AM** 2006 Endocrine disruptors and reproductive health: the case of bisphenol-A. Mol Cell Endocrinol 254–255:179–186
310. **Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM** 2005 Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. Biol Reprod 72:1344–1351
311. **Yoshino S, Yamaki K, Li X, Sai T, Yanagisawa R, Takano H, Taneda S, Hayashi H, Mori Y** 2004 Prenatal exposure to bisphenol A up-regulates immune responses, including

T helper 1 and T helper 2 responses, in mice. Immunology 112:489–495
312. **Yoshino S, Yamaki K, Yanagisawa R, Takano H, Hayashi H, Mori Y** 2003 Effects of bisphenol A on antigen-specific antibody production, proliferative responses of lymphoid cells, and TH1 and TH2 immune responses in mice. Br J Pharmacol 138:1271–1276
313. **Alonso-Magdalena P, Ropero AB, Soriano S, Quesada I, Nadal A** 2010 Bisphenol-A: a new diabetogenic factor? Hormones (Athens) 9:118–126
314. **Rubin BS, Soto AM** 2009 Bisphenol A: perinatal exposure and body weight. Mol Cell Endocrinol 304:55–62
315. **Al-Hiyasat AS, Darmani H, Elbetieha AM** 2002 Effects of bisphenol A on adult male mouse fertility. Eur J Oral Sci 110:163–167
316. **Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, Gadbois JL, Tharp AP, Whitt GS, Sonnenschein C, Soto AM** 2011 Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. Environ Health Perspect 119:547–552
317. **Al-Hiyasat AS, Darmani H, Elbetieha AM** 2004 Leached components from dental composites and their effects on fertility of female mice. Eur J Oral Sci 112:267–272
318. **Salian S, Doshi T, Vanage G** 2009 Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinatally exposed to Bisphenol A. Life Sci 85:11–18
319. **Rubin BS** 2011 Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J Steroid Biochem Mol Biol 127:27–34
320. **Battaglin WA, Rice KC, Focazio MJ, Salmons S, Barry RX** 2009 The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005–2006. Environ Monit Assess 155:281–307
321. **Battaglin WA, Furlong ET, Burkhardt MR, Peter CJ** 2000 Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998. Sci Total Environ 248:123–133
322. **Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TM, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall Jr LW, Williams W** 1996 Ecological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem 15:31–76
323. **Benachour N, Moslemi S, Sipahutar H, Seralini GE** 2007 Cytotoxic effects and aromatase inhibition by xenobiotic endocrine disrupters alone and in combination. Toxicol Appl Pharmacol 222:129–140
324. **Sanderson JT, Seinen W, Giesy JP, van den Berg M** 2000 2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity? Toxicol Sci 54:121–127
325. **Sanderson JT, Letcher RJ, Heneweer M, Giesy JP, van den Berg M** 2001 Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. Environ Health Perspect 109:1027–1031
326. **Hayes TB, Anderson LL, Beasley VR, de Solla SR, Iguchi T, Ingraham H, Kestemont P, Kniewald J, Kniewald Z, Langlois VS, Luque EH, McCoy KA, Muñoz-de-Toro M, Oka T, Oliveira CA, Orton F, Ruby S, Suzawa M, Tavera-Mendoza LE, Trudeau VL, Victor-Costa AB, Willingham E** 2011 Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. J Steroid Biochem Mol Biol 127:64–73
327. **Cooper RL, Laws SC, Das PC, Narotsky MG, Goldman JM, Lee Tyrey E, Stoker TE** 2007 Atrazine and reproductive function: mode and mechanism of action studies. Birth Defects Res B Dev Reprod Toxicol 80:98–112
328. **Stoker TE, Robinette CL, Cooper RL** 1999 Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. Toxicol Sci 52:68–79
329. **Laws SC, Hotchkiss M, Ferrell J, Jayaraman S, Mills L, Modic W, Tinfo N, Fraites M, Stoker T, Cooper R** 2009 Chlorotriazine herbicides and metabolites activate an ACTH-dependent release of corticosterone in male Wistar rats. Toxicol Sci 112:78–87
330. **Fraites MJ, Cooper RL, Buckalew A, Jayaraman S, Mills L, Laws SC** 2009 Characterization of the hypothalamic-pituitary-adrenal axis response to atrazine and metabolites in the female rat. Toxicol Sci 112:88–99
331. **Yoshimoto S, Okada E, Umemoto H, Tamura K, Uno Y, Nishida-Umehara C, Matsuda Y, Takamatsu N, Shiba T, Ito M** 2008 A W-linked DM-domain gene, DM-W, participates in primary ovary development in *Xenopus laevis*. Proc Natl Acad Sci USA 105:2469–2474
332. **Hayes TB** 1998 Sex determination and primary sex differentiation in amphibians. J Exp Zool 281:373–399
333. **Ochoa-Acuña H, Frankenberger J, Hahn L, Carbajo C** 2009 Drinking-water herbicide exposure in Indiana and prevalence of small-for-gestational-age and preterm delivery. Environ Health Perspect 117:1619–1624
334. **Morgan MK, Scheuerman PR, Bishop CS, Pyles RA** 1996 Teratogenic potential of atrazine and 2,4-D using FETAX. J Toxicol Environ Health 48:151–168
335. **Allran JW, Karasov WH** 2001 Effects of atrazine on embryos, larvae, and adults of anuran amphibians. Environ Toxicol Chem 20:769–775
336. **Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, Vonk A** 2002 Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc Natl Acad Sci USA 99:5476–5480
337. **Hayes TB, Khoury V, Narayan A, Nazir M, Park A, Brown T, Adame L, Chan E, Buchholz D, Stueve T, Gallipeau S** 2010 Atrazine induces complete feminization and chemical castration in male African clawed frogs (*Xenopus laevis*). Proc Natl Acad Sci USA 107:4612–4617
338. **Hayes TB, Stuart AA, Mendoza M, Collins A, Noriega N, Vonk A, Johnston G, Liu R, Kpodzo D** 2006 Characterization of atrazine-induced gonadal malformations in African clawed frogs (*Xenopus laevis*) and comparisons with effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17$\beta$-estradiol): support for the demasculinization/feminization hypothesis. Environ Health Perspect 114:134–141
339. **Storrs-Méndez SI, Semlitsch RD** 2010 Intersex gonads in frogs: understanding the time course of natural develop-

ment and role of endocrine disruptors. J Exp Zool B Mol Dev Evol 314:57–66
340. **Carr JA, Gentles A, Smith EE, Goleman WL, Urquidi LJ, Thuett K, Kendall RJ, Giesy JP, Gross TS, Solomon KR, Van Der Kraak G** 2003 Response of larval *Xenopus laevis* to atrazine: assessment of growth, metamorphosis, and gonadal and laryngeal morphology. Environ Toxicol Chem 22:396–405
341. **Hecker M, Kim WJ, Park JW, Murphy MB, Villeneuve D, Coady KK, Jones PD, Solomon KR, Van Der Kraak G, Carr JA, Smith EE, du Preez L, Kendall RJ, Giesy JP** 2005 Plasma concentrations of estradiol and testosterone, gonadal aromatase activity and ultrastructure of the testis in *Xenopus laevis* exposed to estradiol or atrazine. Aquat Toxicol 72: 383–396
342. **Orton F, Carr JA, Handy RD** 2006 Effects of nitrate and atrazine on larval development and sexual differentiation in the northern leopard frog *Rana pipiens*. Environ Toxicol Chem 25:65–71
343. **Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A** 2003 Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. Environ Health Perspect 111:568–575
344. **Tavera-Mendoza L, Ruby S, Brousseau P, Fournier M, Cyr D, Marcogliese D** 2002 Response of the amphibian tadpole (*Xenopus laevis*) to atrazine during sexual differentiation of the testis. Environ Toxicol Chem 21:527–531
345. **Oka T, Tooi O, Mitsui N, Miyahara M, Ohnishi Y, Takase M, Kashiwagi A, Shinkai T, Santo N, Iguchi T** 2008 Effect of atrazine on metamorphosis and sexual differentiation in *Xenopus laevis*. Aquat Toxicol 87:215–226
346. **Langlois VS, Carew AC, Pauli BD, Wade MG, Cooke GM, Trudeau VL** 2010 Low levels of the herbicide atrazine alter sex ratios and reduce metamorphic success in *Rana pipiens* tadpoles raised in outdoor mesocosms. Environ Health Perspect 118:552–557
347. **Jooste AM, Du Preez LH, Carr JA, Giesy JP, Gross TS, Kendall RJ, Smith EE, Van der Kraak GL, Solomon KR** 2005 Gonadal development of larval male *Xenopus laevis* exposed to atrazine in outdoor microcosms. Environ Sci Technol 39:5255–5261
348. **Spolyarich N, Hyne R, Wilson S, Palmer C, Byrne M** 2010 Growth, development and sex ratios of spotted marsh frog (*Limnodynastes tasmaniensis*) larvae exposed to atrazine and a herbicide mixture. Chemosphere 78:807–813
349. **Hecker M, Park JW, Murphy MB, Jones PD, Solomon KR, Van Der Kraak G, Carr JA, Smith EE, du Preez L, Kendall RJ, Giesy JP** 2005 Effects of atrazine on CYP19 gene expression and aromatase activity in testes and on plasma sex steroid concentrations of male African clawed frogs (*Xenopus laevis*). Toxicol Sci 86:273–280
350. **Du Preez LH, Kunene N, Everson GJ, Carr JA, Giesy JP, Gross TS, Hosmer AJ, Kendall RJ, Smith EE, Solomon KR, Van Der Kraak GJ** 2008 Reproduction, larval growth, and reproductive development in African clawed frogs (*Xenopus laevis*) exposed to atrazine. Chemosphere 71:546–552
351. **Kloas W, Lutz I, Springer T, Krueger H, Wolf J, Holden L, Hosmer A** 2009 Does atrazine influence larval development and sexual differentiation in *Xenopus laevis*? Toxicol Sci 107:376–384
352. **U.S. Environmental Protection Agency** 2010 October 9–12, 2007: The potential for atrazine to affect amphibian gonadal development. FIFRA Scientific Advisory Panel Meeting, Arlington, VA, 2007
353. **McDaniel TV, Martin PA, Struger J, Sherry J, Marvin CH, McMaster ME, Clarence S, Tetreault G** 2008 Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (*Rana pipiens*) and green frogs (Rana clamitans) from areas of intensive row crop agriculture. Aquat Toxicol 88:230–242
354. **Reeder AL, Foley GL, Nichols DK, Hansen LG, Wikoff B, Faeh S, Eisold J, Wheeler MB, Warner R, Murphy JE, Beasley VR** 1998 Forms and prevalence of intersexuality and effects of environmental contaminants on sexuality in cricket frogs (*Acris crepitans*). Environ Health Perspect 106:261–266
355. **Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A** 2002 Feminization of male frogs in the wild. Nature 419:895–896
356. **Spolyarich N, Hyne RV, Wilson SP, Palmer CG, Byrne M** 2011 Morphological abnormalities in frogs from a rice-growing region in NSW, Australia, with investigations into pesticide exposure. Environ Monit Assess 173:397–407
357. **Du Preez LH, Kunene N, Hanner R, Giesy JP, Solomon KR, Hosmer A, Van Der Kraak GJ** 2009 Population-specific incidence of testicular ovarian follicles in *Xenopus laevis* from South Africa: a potential issue in endocrine testing. Aquat Toxicol 95:10–16
358. **Murphy MB, Hecker M, Coady KK, Tompsett AR, Jones PD, Du Preez LH, Everson GJ, Solomon KR, Carr JA, Smith EE, Kendall RJ, Van Der Kraak G, Giesy JP** 2006 Atrazine concentrations, gonadal gross morphology and histology in ranid frogs collected in Michigan agricultural areas. Aquat Toxicol 76:230–245
359. **Suzawa M, Ingraham HA** 2008 The herbicide atrazine activates endocrine gene networks via non-steroidal NR5A nuclear receptors in fish and mammalian cells. PLoS ONE 3:e2117
360. **Forson D, Storfer A** 2006 Effects of atrazine and iridovirus infection on survival and life-history traits of the long-toed salamander (*Ambystoma macrodactylum*). Environ Toxicol Chem 25:168–173
361. **Forson DD, Storfer A** 2006 Atrazine increases ranavirus susceptibility in the tiger salamander, Ambystoma tigrinum. Ecol Appl 16:2325–2332
362. **Rohr JR, Palmer BD** 2005 Aquatic herbicide exposure increases salamander desiccation risk eight months later in a terrestrial environment. Environ Toxicol Chem 24:1253–1258
363. **Storrs SI, Kiesecker JM** 2004 Survivorship patterns of larval amphibians exposed to low concentrations of atrazine. Environ Health Perspect 112:1054–1057
364. **Nieves-Puigdoller K, Björnsson BT, McCormick SD** 2007 Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. Aquat Toxicol 84:27–37
365. **Barr DB, Panuwet P, Nguyen JV, Udunka S, Needham LL** 2007 Assessing exposure to atrazine and its metabolites using biomonitoring. Environ Health Perspect 115:1474–1478
366. **Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H, Reynolds SJ, Alavanja MC** 2007 Pesticide

dose estimates for children of Iowa farmers and non-farmers. Environ Res 105:307–315
367. **Rayner JL, Enoch RR, Fenton SE** 2005 Adverse effects of prenatal exposure to atrazine during a critical period of mammary gland growth. Toxicol Sci 87:255–266
368. **Rayner JL, Wood C, Fenton SE** 2004 Exposure parameters necessary for delayed puberty and mammary gland development in Long-Evans rats exposed *in utero* to atrazine. Toxicol Appl Pharmacol 195:23–34
369. **Cooper RL, Stoker TE, Goldman JM, Parrish MB, Tyrey L** 1996 Effect of atrazine on ovarian function in the rat. Reprod Toxicol 10:257–264
370. **Friedmann AS** 2002 Atrazine inhibition of testosterone production in rat males following peripubertal exposure. Reprod Toxicol 16:275–279
371. **Rayner JL, Enoch RR, Wolf DC, Fenton SE** 2007 Atrazine-induced reproductive tract alterations after transplacental and/or lactational exposure in male Long-Evans rats. Toxicol Appl Pharmacol 218:238–248
372. **Karrow NA, McCay JA, Brown RD, Musgrove DL, Guo TL, Germolec DR, White Jr KL** 2005 Oral exposure to atrazine modulates cell-mediated immune function and decreases host resistance to the B16F10 tumor model in female B6C3F1 mice. Toxicology 209:15–28
373. **Enoch RR, Stanko JP, Greiner SN, Youngblood GL, Rayner JL, Fenton SE** 2007 Mammary gland development as a sensitive end point after acute prenatal exposure to an atrazine metabolite mixture in female Long-Evans rats. Environ Health Perspect 115:541–547
374. **Stanko JP, Enoch RR, Rayner JL, Davis CC, Wolf DC, Malarkey DE, Fenton SE** 2010 Effects of prenatal exposure to a low dose atrazine metabolite mixture on pubertal timing and prostate development of male Long-Evans rats. Reprod Toxicol 30:540–549
375. **Schecter A, Birnbaum L, Ryan JJ, Constable JD** 2006 Dioxins: an overview. Environ Res 101:419–428
376. **Mukerjee D** 1998 Health impact of polychlorinated dibenzo-*p*-dioxins: a critical review. J Air Waste Manag Assoc 48:157–165
377. **Emond C, Birnbaum LS, DeVito MJ** 2006 Use of a physiologically based pharmacokinetic model for rats to study the influence of body fat mass and induction of CYP1A2 on the pharmacokinetics of TCDD. Environ Health Perspect 114:1394–1400
378. **Milbrath MO, Wenger Y, Chang CW, Emond C, Garabrant D, Gillespie BW, Jolliet O** 2009 Apparent half-lives of dioxins, furans, and polychlorinated biphenyls as a function of age, body fat, smoking status, and breast-feeding. Environ Health Perspect 117:417–425
379. **Emond C, Michalek JE, Birnbaum LS, DeVito MJ** 2005 Comparison of the use of a physiologically based pharmacokinetic model and a classical pharmacokinetic model for dioxin exposure assessments. Environ Health Perspect 113:1666–1668
380. **Gierthy JF, Crane D** 1984 Reversible inhibition of *in vitro* epithelial cell proliferation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Appl Pharmacol 74:91–98
381. **Korkalainen M, Tuomisto J, Pohjanvirta R** 2001 The AH receptor of the most dioxin-sensitive species, guinea pig, is highly homologous to the human AH receptor. Biochem Biophys Res Commun 285:1121–1129
382. **Okey AB, Riddick DS, Harper PA** 1994 The Ah receptor: mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Toxicol Lett 70: 1–22
383. **Matsumura F** 2009 The significance of the nongenomic pathway in mediating inflammatory signaling of the dioxin-activated Ah receptor to cause toxic effects. Biochem Pharmacol 77:608–626
384. **Birnbaum LS, Tuomisto J** 2000 Non-carcinogenic effects of TCDD in animals. Food Addit Contam 17:275–288
385. **DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA** 1995 Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. Environ Health Perspect 103: 820–831
386. **Kung T, Murphy KA, White LA** 2009 The aryl hydrocarbon receptor (AhR) pathway as a regulatory pathway for cell adhesion and matrix metabolism. Biochem Pharmacol 77:536–546
387. **Li H, Wang H** 2010 Activation of xenobiotic receptors: driving into the nucleus. Expert Opin Drug Metab Toxicol 6:409–426
388. **Marinkoviæ N, Pašaliæ D, Ferenèak G, Gršković B, Stavljeniæ Rukavina A** 2010 Dioxins and human toxicity. Arh Hig Rada Toksikol 61:445–453
389. **White SS, Birnbaum LS** 2009 An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27:197–211
390. **Swedenborg E, Pongratz I** 2010 AhR and ARNT modulate ER signaling. Toxicology 268:132–138
391. **Schwetz BA, Norris JM, Sparschu GL, Rowe UK, Gehring PJ, Emerson JL, Gerbig CG** 1973 Toxicology of chlorinated dibenzo-*p*-dioxins. Environ Health Perspect 5:87–99
392. **Kociba RJ, Schwetz BA** 1982 Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Drug Metab Rev 13: 387–406
393. **Couture LA, Abbott BD, Birnbaum LS** 1990 A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: recent advances toward understanding the mechanism. Teratology 42:619–627
394. **Mocarelli P, Needham LL, Marocchi A, Patterson DG Jr, Brambilla P, Gerthoux PM, Meazza L, Carreri V** 1991 Serum concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and test results from selected residents of Seveso, Italy. J Toxicol Environ Health 32:357–366
395. **Geusau A, Abraham K, Geissler K, Sator MO, Stingl G, Tschachler E** 2001 Severe 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) intoxication: clinical and laboratory effects. Environ Health Perspect 109:865–869
396. **Pohjanvirta R, Tuomisto J** 1994 Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals: effects, mechanisms, and animal models. Pharmacol Rev 46:483–549
397. **Chahoud I, Hartmann J, Rune GM, Neubert D** 1992 Reproductive toxicity and toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3. Effects of single doses on the testis of male rats. Arch Toxicol 66:567–572
398. **Mocarelli P, Gerthoux PM, Needham LL, Patterson Jr DG,**

**Limonta G, Falbo R, Signorini S, Bertona M, Crespi C, Sarto C, Scott PK, Turner WE, Brambilla P** 2011 Perinatal exposure to low doses of dioxin can permanently impair human semen quality. Environ Health Perspect 119:713–718

399. **Mocarelli P, Gerthoux PM, Patterson Jr DG, Milani S, Limonta G, Bertona M, Signorini S, Tramacere P, Colombo L, Crespi C, Brambilla P, Sarto C, Carreri V, Sampson EJ, Turner WE, Needham LL** 2008 Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environ Health Perspect 116:70–77
400. **Foster WG, Maharaj-Briceño S, Cyr DG** 2010 Dioxin-induced changes in epididymal sperm count and spermatogenesis. Environ Health Perspect 118:458–464
401. **Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S** 2010 Interpretation of studies on the developmental reproductive toxicology of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male offspring. Food Chem Toxicol 48:1439–1447
402. **Bjerke DL, Peterson RE** 1994 Reproductive toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male rats: different effects of *in utero* versus lactational exposure. Toxicol Appl Pharmacol 127:241–249
403. **Faqi AS, Dalsenter PR, Merker HJ, Chahoud I** 1998 Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male offspring rats exposed throughout pregnancy and lactation. Toxicol Appl Pharmacol 150:383–392
404. **Gray Jr LE, Kelce WR, Monosson E, Ostby JS, Birnbaum LS** 1995 Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. Toxicol Appl Pharmacol 131: 108–118
405. **Gray LE, Ostby JS, Kelce WR** 1997 A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male Long Evans hooded rat offspring. Toxicol Appl Pharmacol 146: 11–20
406. **Ohsako S, Miyabara Y, Sakaue M, Ishimura R, Kakeyama M, Izumi H, Yonemoto J, Tohyama C** 2002 Developmental stage-specific effects of perinatal 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure on reproductive organs of male rat offspring. Toxicol Sci 66:283–292
407. **Simanainen U, Haavisto T, Tuomisto JT, Paranko J, Toppari J, Tuomisto J, Peterson RE, Viluksela M** 2004 Pattern of male reproductive system effects after *in utero* and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure in three differentially TCDD-sensitive rat lines. Toxicol Sci 80:101–108
408. **Sommer RJ, Ippolito DL, Peterson RE** 1996 *In utero* and lactational exposure of the male Holtzman rat to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: decreased epididymal and ejaculated sperm numbers without alterations in sperm transit rate. Toxicol Appl Pharmacol 140:146–153
409. **Mably TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A, Peterson RE** 1992 *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3. Effects on spermatogenesis and reproductive capability. Toxicol Appl Pharmacol 114:118–126
410. **Wilker C, Johnson L, Safe S** 1996 Effects of developmental exposure to indole-3-carbinol or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on reproductive potential of male rat offspring. Toxicol Appl Pharmacol 141:68–75
411. **Jin MH, Hong CH, Lee HY, Kang HJ, Han SW** 2010 Toxic effects of lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on development of male reproductive system: involvement of antioxidants, oxidants, and p53 protein. Environ Toxicol 25:1–8
412. **Loeffler IK, Peterson RE** 1999 Interactive effects of TCDD and p,p′-DDE on male reproductive tract development in *in utero* and lactationally exposed rats. Toxicol Appl Pharmacol 154:28–39
413. **Rebourcet D, Odet F, Vérot A, Combe E, Meugnier E, Pesenti S, Leduque P, Déchaud H, Magre S, Le Magueresse-Battistoni B** 2010 The effects of an *in utero* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on male reproductive function: identification of Ccl5 as a potential marker. Int J Androl 33:413–424
414. **Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S** 2007 Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the developing male Wistar(Han) rat. I. No decrease in epididymal sperm count after a single acute dose. Toxicol Sci 99:214–223
415. **Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S** 2007 Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the developing male Wistar(Han) rat. II. Chronic dosing causes developmental delay. Toxicol Sci 99:224–233
416. **Ohsako S, Miyabara Y, Nishimura N, Kurosawa S, Sakaue M, Ishimura R, Sato M, Takeda K, Aoki Y, Sone H, Tohyama C, Yonemoto J** 2001 Maternal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) suppressed the development of reproductive organs of male rats: dose-dependent increase of mRNA levels of 5$\alpha$-reductase type 2 in contrast to decrease of androgen receptor in the pubertal ventral prostate. Toxicol Sci 60:132–143
417. **Yonemoto J, Ichiki T, Takei T, Tohyama C** 2005 Maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and the body burden in offspring of Long-Evans rats. Environ Health Prev Med 10:21–32
418. **Arima A, Kato H, Ooshima Y, Tateishi T, Inoue A, Muneoka A, Ihara T, Kamimura S, Fukusato T, Kubota S, Sumida H, Yasuda M** 2009 *In utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces a reduction in epididymal and ejaculated sperm number in rhesus monkeys. Reprod Toxicol 28:495–502
419. **Yamano Y, Asano A, Ohta M, Hirata S, Shoda T, Ohyama K** 2009 Expression of rat sperm flagellum-movement associated protein genes under 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treatment. Biosci Biotechnol Biochem 73:946–949
420. **Korkalainen M, Tuomisto J, Pohjanvirta R** 2004 Primary structure and inducibility by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) of aryl hydrocarbon receptor repressor in a TCDD-sensitive and a TCDD-resistant rat strain. Biochem Biophys Res Commun 315:123–131

421. **Ishimaru N, Takagi A, Kohashi M, Yamada A, Arakaki R, Kanno J, Hayashi Y** 2009 Neonatal exposure to low-dose 2,3,7,8-tetrachlorodibenzo-*p*-dioxin causes autoimmunity due to the disruption of T cell tolerance. J Immunol 182:6576–6586
422. **Nohara K, Fujimaki H, Tsukumo S, Ushio H, Miyabara Y, Kijima M, Tohyama C, Yonemoto J** 2000 The effects of perinatal exposure to low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on immune organs in rats. Toxicology 154:123–133
423. **Lim J, DeWitt JC, Sanders RA, Watkins 3rd JB, Henshel DS** 2007 Suppression of endogenous antioxidant enzymes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced oxidative stress in chicken liver during development. Arch Environ Contam Toxicol 52:590–595
424. **Slezak BP, Hatch GE, DeVito MJ, Diliberto JJ, Slade R, Crissman K, Hassoun E, Birnbaum LS** 2000 Oxidative stress in female B6C3F1 mice following acute and subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicol Sci 54:390–398
425. **Hassoun EA, Wilt SC, Devito MJ, Van Birgelen A, Alsharif NZ, Birnbaum LS, Stohs SJ** 1998 Induction of oxidative stress in brain tissues of mice after subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Sci 42: 23–27
426. **Hermsen SA, Larsson S, Arima A, Muneoka A, Ihara T, Sumida H, Fukusato T, Kubota S, Yasuda M, Lind PM** 2008 *In utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) affects bone tissue in rhesus monkeys. Toxicology 253:147–152
427. **Keller JM, Huet-Hudson Y, Leamy LJ** 2008 Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on molar development among non-resistant inbred strains of mice: a geometric morphometric analysis. Growth Dev Aging 71: 3–16
428. **Kakeyama M, Sone H, Tohyama C** 2008 Perinatal exposure of female rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induces central precocious puberty in the offspring. J Endocrinol 197:351–358
429. **Shi Z, Valdez KE, Ting AY, Franczak A, Gum SL, Petroff BK** 2007 Ovarian endocrine disruption underlies premature reproductive senescence following environmentally relevant chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biol Reprod 76:198–202
430. **Gray LE, Wolf C, Mann P, Ostby JS** 1997 *In utero* exposure to low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alters reproductive development of female Long Evans hooded rat offspring. Toxicol Appl Pharmacol 146:237–244
431. **Jenkins S, Rowell C, Wang J, Lamartiniere CA** 2007 Prenatal TCDD exposure predisposes for mammary cancer in rats. Reprod Toxicol 23:391–396
432. **Mitsui T, Sugiyama N, Maeda S, Tohyama C, Arita J** 2006 Perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin suppresses contextual fear conditioning-accompanied activation of cyclic AMP response element-binding protein in the hippocampal CA1 region of male rats. Neurosci Lett 398:206–210
433. **Seo BW, Powers BE, Widholm JJ, Schantz SL** 2000 Radial arm maze performance in rats following gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Neurotoxicol Teratol 22:511–519
434. **Uemura H, Arisawa K, Hiyoshi M, Kitayama A, Takami H, Sawachika F, Dakeshita S, Nii K, Satoh H, Sumiyoshi Y, Morinaga K, Kodama K, Suzuki T, Nagai M, Suzuki T** 2009 Prevalence of metabolic syndrome associated with body burden levels of dioxin and related compounds among Japan's general population. Environ Health Perspect 117:568–573
435. **Hites RA** 2011 Dioxins: an overview and history. Environ Sci Technol 45:16–20
436. **De Groef B, Decallonne BR, Van der Geyten S, Darras VM, Bouillon R** 2006 Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. Eur J Endocrinol 155:17–25
437. **Blount BC, Valentin-Blasini L, Osterloh JD, Mauldin JP, Pirkle JL** 2007 Perchlorate exposure of the US Population, 2001–2002. J Expo Sci Environ Epidemiol 17:400–407
438. **Greer MA, Goodman G, Pleus RC, Greer SE** 2002 Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans. Environ Health Perspect 110: 927–937
439. **Murray CW, Egan SK, Kim H, Beru N, Bolger PM** 2008 US Food and Drug Administration's Total Diet Study: Dietary intake of perchlorate and iodine. J Expo Sci Environ Epidemiol 18:571–580
440. **Huber DR, Blount BC, Mage DT, Letkiewicz FJ, Kumar A, Allen RH** 2011 Estimating perchlorate exposure from food and tap water based on US biomonitoring and occurrence data. J Expo Sci Environ Epidemiol 21:395–407
441. **Urbansky ET** 2002 Perchlorate as an environmental contaminant. Environ Sci Pollut Res Int 9:187–192
442. **Ginsberg GL, Hattis DB, Zoeller RT, Rice DC** 2007 Evaluation of the U.S. EPA/OSWER preliminary remediation goal for perchlorate in groundwater: focus on exposure to nursing infants. Environ Health Perspect 115:361–369
443. **Dasgupta PK, Dyke JV, Kirk AB, Jackson WA** 2006 Perchlorate in the United States. Analysis of relative source contributions to the food chain. Environ Sci Technol 40: 6608–6614
444. **Tan K, Anderson TA, Jones MW, Smith PN, Jackson WA** 2004 Accumulation of perchlorate in aquatic and terrestrial plants at a field scale. J Environ Qual 33:1638–1646
445. **Miller MD, Crofton KM, Rice DC, Zoeller RT** 2009 Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. Environ Health Perspect 117: 1033–1041
446. **Wolff J** 1998 Perchlorate and the thyroid gland. Pharmacol Rev 50:89–105
447. **Carrasco N** 2000 Thyroid iodide transport: the $Na^+/I^-$ symporter (NIS). In: Braverman LE, Utiger RD, eds. The thyroid: a fundamental and clinical text. 8th ed. Philidelphia: Lippincott, Williams and Wilkins; 52–61
448. **Nicola JP, Basquin C, Portulano C, Reyna-Neyra A, Paroder M, Carrasco N** 2009 The $Na^+/I^-$ symporter mediates active iodide uptake in the intestine. Am J Physiol Cell Physiol 296:C654–C662
449. **Vayre L, Sabourin JC, Caillou B, Ducreux M, Schlumberger M, Bidart JM** 1999 Immunohistochemical analysis

of $Na^+/I^-$ symporter distribution in human extra-thyroidal tissues. Eur J Endocrinol 141:382–386
450. 2007 The $Na^+/I$ symporter (NIS) mediates electroneutral active transport of the environmental pollutant perchlorate. Proc Natl Acad Sci USA 104:20250–20255
451. **Dohan O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, Ginter CS, Carrasco N** 2003 The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. Endocr Rev 24:48–77
452. **Mitchell AM, Manley SW, Morris JC, Powell KA, Bergert ER, Mortimer RH** 2001 Sodium iodide symporter (NIS) gene expression in human placenta. Placenta 22:256–258
453. **Szinnai G, Lacroix L, Carré A, Guimiot F, Talbot M, Martinovic J, Delezoide AL, Vekemans M, Michiels S, Caillou B, Schlumberger M, Bidart JM, Polak M** 2007 Sodium/iodide symporter (NIS) gene expression is the limiting step for the onset of thyroid function in the human fetus. J Clin Endocrinol Metab 92:70–76
454. **Blount BC, Rich DQ, Valentin-Blasini L, Lashley S, Ananth CV, Murphy E, Smulian JC, Spain BJ, Barr DB, Ledoux T, Hore P, Robson M** 2009 Perinatal exposure to perchlorate. thiocyanate, and nitrate in New Jersey mothers and newborns. Environ Sci Technol 43:7543–7549
455. **Blount BC, Valentin-Blasini L** 2006 Analysis of perchlorate, thiocyanate, nitrate and iodide in human amniotic fluid using ion chromatography and electrospray tandem mass spectrometry. Anal Chim Acta 567:87–93
456. **Borjan M, Marcella S, Blount B, Greenberg M, Zhang JJ, Murphy E, Valentin-Blasini L, Robson M** 2011 Perchlorate exposure in lactating women in an urban community in New Jersey. Sci Total Environ 409:460–464
457. **Schier JG, Wolkin AF, Valentin-Blasini L, Belson MG, Kieszak SM, Rubin CS, Blount BC** 2010 Perchlorate exposure from infant formula and comparisons with the perchlorate reference dose. J Expo Sci Environ Epidemiol 20: 281–287
458. **Pearce EN, Leung AM, Blount BC, Bazrafshan HR, He X, Pino S, Valentin-Blasini L, Braverman LE** 2007 Breast milk iodine and perchlorate concentrations in lactating Boston-area women. J Clin Endocrinol Metab 92:1673–1677
459. **Kirk AB, Dyke JV, Martin CF, Dasgupta PK** 2007 Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk. Environ Health Perspect 115:182–186
460. **Zoeller RT, Rovet J** 2004 Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J Neuroendocrinol 16:809–818
461. **Ghassabian A, Bongers-Schokking JJ, Henrichs J, Jaddoe VW, Visser TJ, Visser W, de Muinck Keizer-Schrama SM, Hooijkaas H, Steegers EA, Hofman A, Verhulst FC, van der Ende J, de Rijke YB, Tiemeier H** 2011 Maternal thyroid function during pregnancy and behavioral problems in the offspring: the generation R study. Pediatr Res 69: 454–459
462. **Ghassabian A, Bongers-Schokking JJ, Henrichs J, Jaddoe VW, Visser TJ, Visser W, de Muinck Keizer-Schrama SM, Hooijkaas H, Steegers EA, Hofman A, Verhulst FC, van den Ende J, de Rijke YB, Tiemeier H** 2011 Maternal thyroid function during pregnancy and parent-report problem behavior of the offspring up to age three years. The Generation R Study. Pediatr Res 69(5 Pt 1):454–459
463. **Murcia M, Rebagliato M, Iñiguez C, Lopez-Espinosa MJ, Estarlich M, Plaza B, Barona-Vilar C, Espada M, Vioque J, Ballester F** 2011 Effect of iodine supplementation during pregnancy on infant neurodevelopment at 1 year of age. Am J Epidemiol 173:804–812
464. **Lawrence J, Lamm S, Braverman LE** 2001 Low dose perchlorate (3 mg daily) and thyroid function. Thyroid 11:295
465. **Lawrence JE, Lamm SH, Pino S, Richman K, Braverman LE** 2000 The effect of short-term low-dose perchlorate on various aspects of thyroid function. Thyroid 10:659–663
466. **Braverman LE, Pearce EN, He X, Pino S, Seeley M, Beck B, Magnani B, Blount BC, Firek A** 2006 Effects of six months of daily low-dose perchlorate exposure on thyroid function in healthy volunteers. J Clin Endocrinol Metab 91:2721–2724
467. **National Research Council** 2005 Health implications of perchlorate ingestion. Washington, DC: National Academies Press
468. **Eskenazi B, Warner M, Marks AR, Samuels S, Gerthoux PM, Vercellini P, Olive DL, Needham L, Patterson Jr D, Mocarelli P** 2005 Serum dioxin concentrations and age at menopause. Environ Health Perspect 113:858–862
469. **Bleys J, Navas-Acien A, Laclaustra M, Pastor-Barriuso R, Menke A, Ordovas J, Stranges S, Guallar E** 2009 Serum selenium and peripheral arterial disease: results from the national health and nutrition examination survey, 2003–2004. Am J Epidemiol 169:996–1003
470. **Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, Webster TF** 2008 Body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. Environ Health 7:27
471. **Brucker-Davis F, Thayer K, Colborn T, Fenichel P** 2002 Perchlorate: low dose exposure and susceptible populations. Thyroid 12:739; author reply 739–740
472. **Gibbs JP, Ahmad R, Crump KS, Houck DP, Leveille TS, Findley JE, Francis M** 1998 Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function. J Occup Environ Med 40:1072–1082
473. **Lamm SH, Braverman LE, Li FX, Richman K, Pino S, Howearth G** 1999 Thyroid health status of ammonium perchlorate workers: a cross-sectional occupational health study. J Occup Environ Med 41:248–260
474. **Braverman LE, He X, Pino S, Cross M, Magnani B, Lamm SH, Kruse MB, Engel A, Crump KS, Gibbs JP** 2005 The effect of perchlorate, thiocyanate, and nitrate on thyroid function in workers exposed to perchlorate long-term. J Clin Endocrinol Metab 90:700–706
475. **Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell KL** 2006 Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. Environ Health Perspect 114:1865–1871
476. **LaFranchi SH, Austin J** 2007 How should we be treating children with congenital hypothyroidism? J Pediatr Endocrinol Metab 20:559–578
477. **Steinmaus C, Miller MD, Howd R** 2007 Impact of smoking and thiocyanate on perchlorate and thyroid hormone associations in the 2001–2002 national health and nutrition examination survey. Environ Health Perspect 115: 1333–1338

478. **Li Z, Li FX, Byrd D, Deyhle GM, Sesser DE, Skeels MR, Lamm SH** 2000 Neonatal thyroxine level and perchlorate in drinking water. J Occup Environ Med 42:200–205
479. **Li FX, Byrd DM, Deyhle GM, Sesser DE, Skeels MR, Katkowsky SR, Lamm SH** 2000 Neonatal thyroid-stimulating hormone level and perchlorate in drinking water. Teratology 62:429–431
480. **Lamm SH, Doemland M** 1999 Has perchlorate in drinking water increased the rate of congenital hypothyroidism? J Occup Environ Med 41:409–411
481. **Téllez Téllez R, Michaud Chacón P, Reyes Abarca C, Blount BC, Van Landingham CB, Crump KS, Gibbs JP** 2005 Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. Thyroid 15:963–975
482. **Buffler PA, Kelsh MA, Lau EC, Edinboro CH, Barnard JC, Rutherford GW, Daaboul JJ, Palmer L, Lorey FW** 2006 Thyroid function and perchlorate in drinking water: an evaluation among California newborns, 1998. Environ Health Perspect 114:798–804
483. **Kelsh MA, Buffler PA, Daaboul JJ, Rutherford GW, Lau EC, Barnard JC, Exuzides AK, Madl AK, Palmer LG, Lorey FW** 2003 Primary congenital hypothyroidism, newborn thyroid function, and environmental perchlorate exposure among residents of a southern California community. J Occup Environ Med 45:1116–1127
484. **Amitai Y, Winston G, Sack J, Wasser J, Lewis M, Blount BC, Valentin-Blasini L, Fisher N, Israeli A, Leventhal A** 2007 Gestational exposure to high perchlorate concentrations in drinking water and neonatal thyroxine levels. Thyroid 17:843–850
485. **Steinmaus C, Miller MD, Smith AH** 2010 Perchlorate in drinking water during pregnancy and neonatal thyroid hormone levels in California. J Occup Environ Med 52: 1217–1524
486. **Brechner RJ, Parkhurst GD, Humble WO, Brown MB, Herman WH** 2000 Ammonium perchlorate contamination of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona. J Occup Environ Med 42:777–782
487. **Crump C, Michaud P, Téllez R, Reyes C, Gonzalez G, Montgomery EL, Crump KS, Lobo G, Becerra C, Gibbs JP** 2000 Does perchlorate in drinking water affect thyroid function in newborns or school-age children? J Occup Environ Med 42:603–612
488. **Pearce EN, Spencer CA, Mestman JH, Lee RH, Bergoglio LM, Mereshian P, He X, Leung AM, Braverman LE** 2011 The effect of environmental perchlorate on thyroid function in pregnant women from Cordoba, Argentina, and Los Angeles, California. Endocr Pract 17:412–417
489. **Pearce EN, Lazarus JH, Smyth PP, He X, Dall'amico D, Parkes AB, Burns R, Smith DF, Maina A, Bestwick JP, Jooman M, Leung AM, Braverman LE** 2010 Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women. J Clin Endocrinol Metab 95: 3207–3215
490. **Gibbs JP, Van Landingham C** 2008 Urinary perchlorate excretion does not predict thyroid function among pregnant women. Thyroid 18:807–808
491. **Zoeller TR** 2010 Environmental chemicals targeting thyroid. Hormones 9:28–40
492. **Fenner-Crisp PA** 2000 Endocrine modulators: risk characterization and assessment. Toxicol Pathol 28:438–440
493. **Lucier GW** 1997 Dose-response relationships for endocrine disruptors: what we know and what we don't know. Regul Toxicol Pharmacol 26:34–35
494. **Sheehan DM, Willingham E, Gaylor D, Bergeron JM, Crews D** 1999 No threshold dose for estradiol-induced sex reversal of turtle embryos: how little is too much? Environ Health Perspect 107:155–159
495. **Sheehan DM, vom Saal FS** 1997 Low dose effects of hormones: a challenge for risk assessment. Risk Policy Report 4:31–39
496. **Crews D, Bergeron JM, McLachlan JA** 1995 The role of estrogen in turtle sex determination and the effect of PCBs. Environ Health Perspect 103(Suppl 7):73–77
497. **vom Saal FS, Sheehan DM** 1998 Challenging risk assessment. Forum Appl Res Public Policy 13:11–18
498. **Bergeron JM, Crews D, McLachlan JA** 1994 PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. Environ Health Perspect 102:780–781
499. **Sonnenschein C, Olea N, Pasanen ME, Soto AM** 1989 Negative controls of cell proliferation: human prostate cancer cells and androgens. Cancer Res 49:3474–3481
500. **Geck P, Szelei J, Jimenez J, Lin TM, Sonnenschein C, Soto AM** 1997 Expression of novel genes linked to the androgen-induced, proliferative shutoff in prostate cancer cells. J Steroid Biochem Mol Biol 63:211–218
501. **Soto AM, Lin TM, Sakabe K, Olea N, Damassa DA, Sonnenschein C** 1995 Variants of the human prostate LNCaP cell line as a tool to study discrete components of the androgen-mediated proliferative response. Oncol Res 7:545–558
502. **Geck P, Maffini MV, Szelei J, Sonnenschein C, Soto AM** 2000 Androgen-induced proliferative quiescence in prostate cancer: the role of AS3 as its mediator. Proc Natl Acad Sci USA 97:10185–10190
503. **Soto AM, Sonnenschein C** 1985 The role of estrogens on the proliferation of human breast tumor cells (MCF-7). J Steroid Biochem 23:87–94
504. **Amara JF, Dannies PS** 1983 17$\beta$-Estradiol has a biphasic effect on GH cell growth. Endocrinology 112:1141–1143
505. **Soto AM, Sonnenschein C** 2001 The two faces of Janus: sex steroids as mediators of both cell proliferation and cell death. J Natl Cancer Inst 93:1673–1675
506. **Sonnenschein C, Soto AM** 2008 Theories of carcinogenesis: an emerging perspective. Semin Cancer Biol 18:372–377
507. **Harris H** 2004 Tumour suppression: putting on the brakes. Nature 427:201
508. **Yusuf I, Fruman DA** 2003 Regulation of quiescence in lymphocytes. Trends Immunol 24:380–386
509. **Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, Cohen P, Smith A** 2008 The ground state of embryonic stem cell self-renewal. Nature 453:519–523
510. **Carroll JS, Meyer CA, Song J, Li W, Geistlinger TR, Eeckhoute J, Brodsky AS, Keeton EK, Fertuck KC, Hall GF, Wang Q, Bekiranov S, Sementchenko V, Fox EA, Silver PA, Gingeras TR, Liu XS, Brown M** 2006 Genome-wide analysis of estrogen receptor binding sites. Nat Genet 38: 1289–1297

511. **Maffini M, Denes V, Sonnenschein C, Soto A, Geck P** 2008 APRIN is a unique Pds5 paralog with features of a chromatin regulator in hormonal differentiation. J Steroid Biochem Mol Biol 108:32–43
512. **Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M, Gustafsson JA** 2007 Estrogen receptors: how do they signal and what are their targets. Physiol Rev 87:905–931
513. **Barkhem T, Nilsson S, Gustafsson JA** 2004 Molecular mechanisms, physiological consequences and pharmacological implications of estrogen receptor action. Am J Pharmacogenomics 4:19–28
514. **Shi YB** 2009 Dual functions of thyroid hormone receptors in vertebrate development: the roles of histone-modifying cofactor complexes. Thyroid 19:987–999
515. **Kang HY, Tsai MY, Chang C, Huang KE** 2003 Mechanisms and clinical relevance of androgens and androgen receptor actions. Chang Gung Med J 26:388–402
516. **Jeyakumar M, Webb P, Baxter JD, Scanlan TS, Katzenellenbogen JA** 2008 Quantification of ligand-regulated nuclear receptor corepressor and coactivator binding, key interactions determining ligand potency and efficacy for the thyroid hormone receptor. Biochemistry 47:7465–7476
517. **Nandi S** 1958 Endocrine control of mammary gland development and function in the C3H/ He Crgl mouse. J Natl Cancer Inst 21:1039–1063
518. **Humphreys RC, Krajewska M, Krnacik S, Jaeger R, Weiher H, Krajewski S, Reed JC, Rosen JM** 1996 Apoptosis in the terminal end bud of the murine mammary gland: a mechanism of ductal morphogenesis. Development 122:4013–4022
519. **Haslam SZ** 1986 Mammary fibroblast influence on normal mouse mammary epithelial cell responses to estrogen in vitro. Cancer Res 46:310–316
520. **McGrath CM** 1983 Augmentation of the response of normal mammary epithelial cells to estradiol by mammary stroma. Cancer Res 43:1355–1360
521. **Sohoni P, Sumpter JP** 1998 Several environmental oestrogens are also anti-androgens. J Endocrinol 158:327–339
522. **Tilghman SL, Nierth-Simpson EN, Wallace R, Burow ME, McLachlan JA** 2010 Environmental hormones: Multiple pathways for response may lead to multiple disease outcomes. Steroids 75:520–523
523. **Ismail A, Nawaz Z** 2005 Nuclear hormone receptor degradation and gene transcription: an update. IUBMB Life 57:483–490
524. **Hoeck W, Rusconi S, Groner B** 1989 Down-regulation and phosphorylation of glucocorticoid receptors in cultured cells. Investigations with a monospecific antiserum against a bacterially expressed receptor fragment. J Biol Chem 264:14396–14402
525. **Lange CA, Shen T, Horwitz KB** 2000 Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. Proc Natl Acad Sci USA 97:1032–1037
526. **Nawaz Z, Lonard DM, Dennis AP, Smith CL, O'Malley BW** 1999 Proteasome-dependent degradation of the human estrogen receptor. Proc Natl Acad Sci USA 96:1858–1862
527. **Lin HK, Altuwaijri S, Lin WJ, Kan PY, Collins LL, Chang C** 2002 Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with coregulators in prostate cancer cells. J Biol Chem 277: 36570–36576
528. **von Zastrow M, Kobilka BK** 1994 Antagonist-dependent and -independent steps in the mechanism of adrenergic receptor internalization. J Biol Chem 269:18448–18452
529. **Modrall JG, Nanamori M, Sadoshima J, Barnhart DC, Stanley JC, Neubig RR** 2001 ANG II type 1 receptor down-regulation does not require receptor endocytosis or G protein coupling. Am J Physiol Cell Physiol 281:C801–C809
530. **Kinyamu HK, Archer TK** 2003 Estrogen receptor-dependent proteasomal degradation of the glucocorticoid receptor is coupled to an increase in mdm2 protein expression. Mol Cell Biol 23:5867–5881
531. **Freedman NJ, Lefkowitz RJ** 1996 Desensitization of G protein-coupled receptors. Recent Prog Horm Res 51: 319–351; discussion 352–353
532. **Lohse MJ** 1993 Molecular mechanisms of membrane receptor desensitization. Biochim Biophys Acta 1179:171–188
533. **Bohm SK, Grady EF, Bunnett NW** 1997 Regulatory mechanisms that modulate signalling by G-protein-coupled receptors. Biochem J 322:1–18
534. **Shankaran H, Wiley HS, Resat H** 2007 Receptor down-regulation and desensitization enhance the information processing ability of signalling receptors. BMC Syst Biol 1:48
535. **Lesser B, Bruchovsky N** 1974 Effect of duration of the period after castration on the response of the rat ventral prostate to androgens. Biochem J 142:429–431
536. **Stormshak F, Leake R, Wertz N, Gorski J** 1976 Stimulatory and inhibitory effects of estrogen on uterine DNA synthesis. Endocrinology 99:1501–1511
537. **Bruchovsky N, Lesser B, Van Doorn E, Craven S** 1975 Hormonal effects on cell proliferation in rat prostate. Vitam Horm 33:61–102
538. **Coser KR, Chesnes J, Hur J, Ray S, Isselbacher KJ, Shioda T** 2003 Global analysis of ligand sensitivity of estrogen inducible and suppressible genes in MCF7/BUS breast cancer cells by DNA microarray. Proc Natl Acad Sci USA 100:13994–13999
539. **Hur J, Chesnes J, Coser KR, Lee RS, Geck P, Isselbacher KJ, Shioda T** 2004 The Bik BH3-only protein is induced in estrogen-starved and antiestrogen-exposed breast cancer cells and provokes apoptosis. Proc Natl Acad Sci USA 101: 2351–2356
540. **Li L, Andersen ME, Heber S, Zhang Q** 2007 Non-monotonic dose-response relationship in steroid hormone receptor-mediated gene expression. J Mol Endocrinol 38:569–585
541. **Vandenberg LN, Wadia PR, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM** 2006 The mammary gland response to estradiol: monotonic at the cellular level, non-monotonic at the tissue-level of organization? J Steroid Biochem Mol Biol 101:263–274
542. **Schell LM, Burnitz KK, Lathrop PW** 2010 Pollution and human biology. Ann Hum Biol 37:347–366
543. **Plotkin D, Lechner JJ, Jung WE, Rosen PJ** 1978 Tamoxifen flare in advanced breast cancer. JAMA 240:2644–2646
544. **Osborne CK, Hobbs K, Clark GM** 1985 Effect of estrogens

and antiestrogens on growth of human breast cancer cells in athymic nude mice. Cancer Res 45:584–590
545. **Berthois Y, Pons M, Dussert C, Crastes de Paulet A, Martin PM** 1994 Agonist-antagonist activity of anti-estrogens in the human breast cancer cell line MCF-7: an hypothesis for the interaction with a site distinct from the estrogen binding site. Mol Cell Endocrinol 99:259–268
546. **Reddel RR, Sutherland RL** 1984 Tamoxifen stimulation of human breast cancer cell proliferation in vitro: a possible model for tamoxifen tumour flare. Eur J Cancer Clin Oncol 20:1419–1424
547. **Wolf DM, Langan-Fahey SM, Parker CJ, McCague R, Jordan VC** 1993 Investigation of the mechanism of tamoxifen-stimulated breast tumor growth with nonisomerizable analogues of tamoxifen and metabolites. J Natl Cancer Inst 85:806–812
548. **Howell A** 2001 Preliminary experience with pure antiestrogens. Clin Cancer Res 7:4369s–4375s; discusion 4411s–4412s
549. **Hattar R, Maller O, McDaniel S, Hansen KC, Hedman KJ, Lyons TR, Lucia S, Wilson Jr RS, Schedin P** 2009 Tamoxifen induces pleiotrophic changes in mammary stroma resulting in extracellular matrix that suppresses transformed phenotypes. Breast Cancer Res 11:R5
550. **Howell A, Landberg G, Bergh J** 2009 Breast tumour stroma is a prognostic indicator and target for therapy. Breast Cancer Res 11(Suppl 3):S16
551. **Langan-Fahey SM, Tormey DC, Jordan VC** 1990 Tamoxifen metabolites in patients on long-term adjuvant therapy for breast cancer. Eur J Cancer 26:883–888
552. **Kuiper GG, van den Bemd GJ, van Leeuwen JP** 1999 Estrogen receptor and the SERM concept. J Endocrinol Invest 22:594–603
553. **MacGregor JI, Jordan VC** 1998 Basic guide to the mechanisms of antiestrogen action. Pharmacol Rev 50:151–196
554. **Grese TA, Dodge JA** 1998 Selective estrogen receptor modulators (SERMs). Curr Pharm Des 4:71–92
555. **Nagel SC, Hagelbarger JL, McDonnell DP** 2001 Development of an ER action indicator mouse for the study of estrogens, selective ER modulators (SERMs), and xenobiotics. Endocrinology 142:4721–4728
556. **Gaido KW, Leonard LS, Lovell S, Gould JC, Babaï D, Portier CJ, McDonnell DP** 1997 Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol Appl Pharmacol 143:205–212
557. **Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, Safe S, McDonnell DP, Gaido KW** 1998 Bisphenol A interacts with the estrogen receptor $\alpha$ in a distinct manner from estradiol. Mol Cell Endocrinol 142: 203–214
558. **Lerner HJ, Band PR, Israel L, Leung BS** 1976 Phase II study of tamoxifen: report of 74 patients with stage IV breast cancer. Cancer Treat Rep 60:1431–1435
559. **Zhang HH, Kumar S, Barnett AH, Eggo MC** 1999 Intrinsic site-specific differences in the expression of leptin in human adipocytes and its autocrine effects on glucose uptake. J Clin Endocrinol Metab 84:2550–2556
560. **Haddad N, Howland R, Baroody G, Daher C** 2006 The modulatory effect of leptin on the overall insulin production in ex-vivo normal rat pancreas. Can J Physiol Pharmacol 84:157–162
561. **Pallett AL, Morton NM, Cawthorne MA, Emilsson V** 1997 Leptin inhibits insulin secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. Biochem Biophys Res Commun 238:267–270
562. **Thorburn AW, Holdsworth A, Proietto J, Morahan G** 2000 Differential and genetically separable associations of leptin with obesity-related traits. Int J Obes Relat Metab Disord 24:742–750
563. **Lieb W, Sullivan LM, Harris TB, Roubenoff R, Benjamin EJ, Levy D, Fox CS, Wang TJ, Wilson PW, Kannel WB, Vasan RS** 2009 Plasma leptin levels and incidence of heart failure, cardiovascular disease, and total mortality in elderly individuals. Diabetes Care 32:612–616
564. **Neel BA, Sargis RM** 2011 The paradox of progress: environmental disruption of metabolism and the diabetes epidemic. Diabetes 60:1838–1848
565. **Sargis RM, Johnson DN, Choudhury RA, Brady MJ** 2010 Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. Obesity (Silver Spring) 18:1283–1288
566. **Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N** 2008 Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. Environ Health Perspect 116:1642–1647
567. **Ben-Jonathan N, Hugo ER, Brandebourg TD** 2009 Effects of bisphenol A on adipokine release from human adipose tissue: implications for the metabolic syndrome. Mol Cell Endocrinol 304:49–54
568. **Miyawaki J, Sakayama K, Kato H, Yamamoto H, Masuno H** 2007 Perinatal and postnatal exposure to bisphenol a increases adipose tissue mass and serum cholesterol level in mice. J Atheroscler Thromb 14:245–252
569. **Botelho GG, Golin M, Bufalo AC, Morais RN, Dalsenter PR, Martino-Andrade AJ** 2009 Reproductive effects of di(2-ethylhexyl)phthalate in immature male rats and its relation to cholesterol, testosterone, and thyroxin levels. Arch Environ Contam Toxicol 57:777–784
570. **Lutz WK, Gaylor DW, Conolly RB, Lutz RW** 2005 Nonlinearity and thresholds in dose-response relationships for carcinogenicity due to sampling variation, logarithmic dose scaling, or small differences in individual susceptibility. Toxicol Appl Pharmacol 207:565–569
571. **Center for the Evaluation of Risks to Human Reproduction** 2007 NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Washington, DC: Department of Health and Human Services
572. **Willhite CC, Ball GL, McLellan CJ** 2008 Derivation of a Bisphenol A organ reference dose (RfD) and drinking-water equivalent concentration. J Toxicol Environ Health B Crit Rev 11:69–146
573. **Sakamoto H, Yokota H, Kibe R, Sayama Y, Yuasa A** 2002 Excretion of bisphenol A-glucuronide into the small intestine and deconjugation in the cecum of the rat. Biochem Biophys Acta 1573:171–176
574. **Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, Faure R, Cravedi JP** 2003 Biotransformations of bisphenol A in a mammalian model: answers and new

questions raised by low-dose metabolic fate studies in pregnant CD1 mice. Environ Health Perspect 111:309–319
575. **Stowell CL, Barvian KK, Young PC, Bigsby RM, Verdugo DE, Bertozzi CR, Widlanski TS** 2006 A role for sulfation-desulfation in the uptake of bisphenol A into breast tumor cells. Chem Biol 13:891–897
576. **Center for the Evaluation of Risks to Human Reproduction** 2008 Bisphenol A: public comments. Washington, DC: Department of Health and Human Services
577. **Markey CM, Michaelson CL, Veson EC, Sonnenschein C, Soto AM** 2001 The mouse uterotrophic assay: a reevaluation of its validity in assessing the estrogenicity of bisphenol A. Environ Health Perspect 109:55–60
578. **Schönfelder G, Friedrich K, Paul M, Chahoud I** 2004 Developmental effects of prenatal exposure to bisphenol A on the uterus of rat offspring. Neoplasia 6:584–594
579. **Eskenazi B, Mocarelli P, Warner M, Needham L, Patterson DG Jr, Samuels S, Turner W, Gerthoux PM, Brambilla P** 2004 Relationship of serum TCDD concentrations and age at exposure of female residents of Seveso, Italy. Environ Health Perspect 112:22–27
580. **Warner M, Eskenazi B, Mocarelli P, Gerthoux PM, Samuels S, Needham L, Patterson D, Brambilla P** 2002 Serum dioxin concentrations and breast cancer risk in the Seveso Women's Health Study. Environ Health Perspect 110: 625–628
581. **Eskenazi B, Mocarelli P, Warner M, Samuels S, Vercellini P, Olive D, Needham LL, Patterson Jr DG, Brambilla P, Gavoni N, Casalini S, Panazza S, Turner W, Gerthoux PM** 2002 Serum dioxin concentrations and endometriosis: a cohort study in Seveso, Italy. Environ Health Perspect 110: 629–634
582. **Eskenazi B, Warner M, Mocarelli P, Samuels S, Needham LL, Patterson DG Jr, Lippman S, Vercellini P, Gerthoux PM, Brambilla P, Olive D** 2002 Serum dioxin concentrations and menstrual cycle characteristics. Am J Epidemiol 156:383–392
583. **Robinson GW, Karpf AB, Kratochwil K** 1999 Regulation of mammary gland development by tissue interaction. J Mammary Gland Biol Neoplasia 4:9–19
584. **Medina D, Sivaraman L, Hilsenbeck SG, Conneely O, Ginger M, Rosen J, Omalle BW** 2001 Mechanisms of hormonal prevention of breast cancer. Ann NY Acad Sci 952: 23–35
585. **Schulz KM, Molenda-Figueira HA, Sisk CL** 2009 Back to the future: the organizational-activational hypothesis adapted to puberty and adolescence. Horm Behav 55:597–604
586. **Schulz KM, Sisk CL** 2006 Pubertal hormones, the adolescent brain, and the maturation of social behaviors: lessons from the Syrian hamster. Mol Cell Endocrinol 254–255: 120–126
587. **Primus RJ, Kellogg CK** 1990 Gonadal hormones during puberty organize environment-related social interaction in the male rat. Horm Behav 24:311–323
588. **Arase S, Ishii K, Igarashi K, Aisaki K, Yoshio Y, Matsushima A, Shimohigashi Y, Arima K, Kanno J, Sugimura Y** 2011 Endocrine disrupter bisphenol A increases in situ estrogen production in the mouse urogenital sinus. Biol Reprod 84:734–742
589. **Lee DH, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs Jr DR** 2010 Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. Environ Health Perspect 118:1235–1242
590. **Lee DH, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs Jr DR** 2011 Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PLoS ONE 6:e15977
591. **Shin JY, Choi YY, Jeon HS, Hwang JH, Kim SA, Kang JH, Chang YS, Jacobs DR Jr, Park JY, Lee DH** 2010 Low-dose persistent organic pollutants increased telomere length in peripheral leukocytes of healthy Koreans. Mutagenesis 25: 511–516
592. **MacLusky NJ, Hajszan T, Leranth C** 2005 The environmental estrogen bisphenol A inhibits estradiol-induced hippocampal synaptogenesis. Environ Health Perspect 113:675–679
593. **Della Seta D, Minder I, Dessì-Fulgheri F, Farabollini F** 2005 Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats. Brain Res Bull 65: 255–260
594. **Razzoli M, Valsecchi P, Palanza P** 2005 Chronic exposure to low doses bisphenol A interferes with pair-bonding and exploration in female Mongolian gerbils. Brain Res Bull 65:249–254
595. **Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A** 2006 The estrogenic effect of bisphenol A disrupts pancreatic $\beta$-cell function in vivo and induces insulin resistance. Environ Health Perspect 114:106–112
596. **Titus-Ernstoff L, Hatch EE, Hoover RN, Palmer J, Greenberg ER, Ricker W, Kaufman R, Noller K, Herbst AL, Colton T, Hartge P** 2001 Long-term cancer risk in women given diethylstilbestrol (DES) during pregnancy. Br J Cancer 84:126–133
597. **Calle EE, Mervis CA, Thun MJ, Rodriguez C, Wingo PA, Heath Jr CW** 1996 Diethylstilbestrol and risk of fatal breast cancer in a prospective cohort of US women. Am J Epidemiol 144:645–652
598. **Small CM, DeCaro JJ, Terrell ML, Dominguez C, Cameron LL, Wirth J, Marcus M** 2009 Maternal exposure to a brominated flame retardant and genitourinary conditions in male offspring. Environ Health Perspect 117:1175–1179
599. **Goldberg JM, Falcone T** 1999 Effect of diethylstilbestrol on reproductive function. Fertil Steril 72:1–7
600. **Hatch EE, Herbst AL, Hoover RN, Noller KL, Adam E, Kaufman RH, Palmer JR, Titus-Ernstoff L, Hyer M, Hartge P, Robboy SJ** 2001 Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States). Cancer Causes Control 12:837–845
601. **Terrell ML, Berzen AK, Small CM, Cameron LL, Wirth JJ, Marcus M** 2009 A cohort study of the association between secondary sex ratio and parental exposure to polybrominated biphenyl (PBB) and polychlorinated biphenyl (PCB). Environ Health 8:35
602. **Xu X, Dailey AB, Talbott EO, Ilacqua VA, Kearney G, Asal NR** 2010 Associations of serum concentrations of organochlorine pesticides with breast cancer and prostate cancer in U.S. adults. Environ Health Perspect 118:60–66
603. **Li DK, Zhou Z, Miao M, He Y, Qing D, Wu T, Wang J,**

**Weng X, Ferber J, Herrinton LJ, Zhu Q, Gao E, Yuan W** 2010 Relationship between urine bisphenol-A level and declining male sexual function. J Androl 31:500–506
604. **Lim JS, Lee DH, Jacobs Jr DR** 2008 Association of brominated flame retardants with diabetes and metabolic syndrome in the U.S. population, 2003–2004. Diabetes Care 31:1802–1807
605. **Giordano F, Abballe A, De Felip E, di Domenico A, Ferro F, Grammatico P, Ingelido AM, Marra V, Marrocco G, Vallasciani S, Figà-Talamanca I** 2010 Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth Defects Res A Clin Mol Teratol 88:241–250
606. **Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J, Calafat AM** 2008 Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect 116:1092–1097
607. **Sunyer J, Garcia-Esteban R, Alvarez M, Guxens M, Goñi F, Basterrechea M, Vrijheid M, Guerra S, Antó JM** 2010 DDE in mothers' blood during pregnancy and lower respiratory tract infections in their infants. Epidemiology 21: 729–735
608. **World Health Organization** 2002 Global assessment of the state-of-the-science of endocrine disruptors. Geneva: World Health Organization
609. **Tyl RW** 2009 Basic exploratory research versus guideline-compliant studies used for hazard evaluation and risk assessment: bisphenol A as a case study. Environ Health Perspect 117:1644–1651
610. **Tyl RW** 2010 In honor of the Teratology Society's 50th anniversary: the role of Teratology Society members in the development and evolution of in vivo developmental toxicity test guidelines. Birth Defects Res C Embryo Today 90:99–102
611. **Rice C, Birnbaum LS, Cogliano J, Mahaffey K, Needham L, Rogan WJ, vom Saal FS** 2003 Exposure assessment for endocrine disruptors: some considerations in the design of studies. Environ Health Perspect 111:1683–1690
612. **Soto AM, Rubin BS, Sonnenschein C** 2009 Interpreting endocrine disruption from an integrative biology perspective. Mol Cell Endocrinol 304:3–7
613. **Heindel JJ** 2008 Animal models for probing the developmental basis of disease and dysfunction paradigm. Basic Clin Pharmacol Toxicol 102:76–81
614. **Heindel JJ, vom Saal FS** 2009 Role of nutrition and environmental endocrine disrupting chemicals during the perinatal period on the aetiology of obesity. Mol Cell Endocrinol 304:90–96
615. **Newbold RR, Padilla-Banks E, Jefferson WN, Heindel JJ** 2008 Effects of endocrine disruptors on obesity. Int J Androl 31:201–208
616. **Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J, Vickers C** 2008 IPCS framework for analyzing the relevance of a noncancer mode of action for humans. Crit Rev Toxicol 38:87–96
617. **German Federal Institute for Risk Assessment (BfR)** 2009 Establishment of assessment and decision criteria in human health risk assessment for substances with endocrine disrupting properties under the EU plan protection product regulation. Report of a workshop hosted at the German Federal Institute for Risk Assessment (BfR), Berlin, Germany, 2009
618. **Lidsky TI, Schneider JS** 2006 Adverse effects of childhood lead poisoning: the clinical neuropsychological perspective. Environ Res 100:284–293
619. **Sheehan DM** 2006 No-threshold dose-response curves for nongenotoxic chemicals: findings and application for risk assessment. Environ Res 100:93–99
620. **Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC** 2009 Endocrine-disrupting chemical: an Endocrine Society scientific statement. Endocr Rev 30:293–342
621. **American Society of Human Genetics; American Society for Reproductive Medicine; Endocrine Society; Genetics Society of America; Society for Developmental Biology; Society for Pediatric Urology; Society for the Study of Reproduction; Society for Gynecologic Investigation** 2011 Assessing chemical risk: societies offer expertise. Science 331:1136
622. **Tominaga T, Negishi T, Hirooka H, Miyachi A, Inoue A, Hayasaka I, Yoshikawa Y** 2006 Toxicokinetics of bisphenol A in rats, monkeys and chimpanzees by the LC-MS/MS method. Toxicology 226:208–217
623. **Newbold RR** 2004 Lessons learned from perinatal exposure to diethylstilbestrol. Toxicol Appl Pharmacol 199: 142–150
624. **Taylor JA, Vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain PL, Laffont CM, VandeVoort CA** 2011 Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. Environ Health Perspect 119:422–430
625. **Gies A, Heinzow B, Dieter HH, Heindel J** 2009 Bisphenol A workshop of the German Federal Government Agency: March 30–31, 2009. Work group report: public health issues of bisphenol A. Int J Hyg Environ Health 212:693–696
626. **World Health Organization** 2010 Joint FAO/WHO expert meeting to review toxicological and health aspects of bisphenol A. Geneva: World Health Organization
627. **Kortenkamp A** 2008 Low dose mixture effects of endocrine disrupters: implications for risk assessment and epidemiology. Int J Androl 31:233–240
628. **Bergeron JM, Willingham E, Osborn CT 3rd, Rhen T, Crews D** 1999 Developmental synergism of steroidal estrogens in sex determination. Environ Health Perspect 107:93–97
629. **Rajapakse N, Silva E, Kortenkamp A** 2002 Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone activity. Environ Health Perspect 110:917–921
630. **Rajapakse N, Silva E, Scholze M, Kortenkamp A** 2004 Deviation from additivity with estrogenic mixtures containing 4-nonylphenol and 4-tert-octylphenol detected in the E-SCREEN assay. Environ Sci Technol 38:6343–6352
631. **Kortenkamp A, Faust M, Scholze M, Backhaus T** 2007 Low-level exposure to multiple chemicals: reason for human health concerns? Environ Health Perspect 115(Suppl 1):106–114
632. **Silins I, Högberg J** 2011 Combined toxic exposures and human health: biomarkers of exposure and effect. Int J Environ Res Public Health 8:629–647
633. **Rudel RA, Gray JM, Engel CL, Rawsthorne TW, Dodson RE, Ackerman JM, Rizzo J, Nudelman JL, Brody JG** 2011

Food packaging and bisphenol A and bis(2-ethylhexyl) phthalate exposure: findings from a dietary intervention. Environ Health Perspect 119:914–920
634. **Ji K, Kho YL, Park Y, Choi K** 2010 Influence of a five-day vegetarian diet on urinary levels of antibiotics and phthalate metabolites: a pilot study with "Temple Stay" participants Environ Res 110:375–382
635. **Carwile JL, Luu HT, Bassett LS, Driscoll DA, Yuan C, Chang JY, Ye X, Calafat AM, Michels KB** 2009 Polycarbonate bottle use and urinary bisphenol A concentrations. Environ Health Perspect 117:1368–1372
636. **Matsumoto A, Kunugita N, Kitagawa K, Isse T, Oyama T, Foureman GL, Morita M, Kawamoto T** 2003 Bisphenol A levels in human urine. Environ Health Perspect 111:101–104
637. **Kawagoshi Y, Fujita Y, Kishi I, Fukunaga I** 2003 Estrogenic chemicals and estrogenic activity in leachate from municipal waste landfill determined by yeast two-hybrid assay. J Environ Monit 5:269–274
638. **Liao C, Kannan K** 2011 High levels of bisphenol a in paper currencies from several countries, and implications for dermal exposure. Environ Sci Technol 45:6761–6768
639. **Lopez-Espinosa MJ, Granada A, Araque P, Molina-Molina JM, Puertollano MC, Rivas A, Fernández M, Cerrillo I, Olea-Serrano MF, López C, Olea N** 2007 Oestrogenicity of paper and cardboard extracts used as food containers. Food Addit Contam 24:95–102
640. **Terasaki M, Shiraishi F, Fukazawa H, Makino M** 2007 Occurrence and estrogenicity of phenolics in paper-recycling process water: pollutants originating from thermal paper in waste paper. Environ Toxicol Chem 26:2356–2366
641. **Carson R** 1962 Silent spring. Boston, MA: Houghton Mifflin
642. **Chung E, Genco MC, Megrelis L, Ruderman JV** 2011 Effects of bisphenol A and triclocarban on brain-specific expression of aromatase in early zebrafish embryos. Proc Natl Acad Sci USA 108:17732–17737
643. **Rhee JS, Kim BM, Lee CJ, Yoon YD, Lee YM, Lee JS** 2011 Bisphenol A modulates expression of sex differentiation genes in the self-fertilizing fish, Kryptolebias marmoratus. Aquat Toxicol 104:218–229
644. **Hatef A, Alavi SM, Abdulfatah A, Fontaine P, Rodina M, Linhart O** 2012 Adverse effects of bisphenol A on reproductive physiology in male goldfish at environmentally relevant concentrations. Ecotoxicol Environ Saf 76:56–62
645. **Bai Y, Zhang YH, Zhai LL, Li XY, Yang J, Hong YY** 2011 Estrogen receptor expression and vitellogenin synthesis induced in hepatocytes of male frogs *Rana chensinensis* exposed to bisphenol A. Zool Res 32:317–322
646. **Levy G, Lutz I, Krüger A, Kloas W** 2004 Bisphenol A induces feminization in *Xenopus laevis* tadpoles. Environ Res 94:102–111
647. **Stoker C, Rey F, Rodriguez H, Ramos JG, Sirosky P, Larriera A, Luque EH, Muñoz-de-Toro M** 2003 Sex reversal effects on *Caiman latirostris* exposed to environmentally relevant doses of the xenoestrogen bisphenol A. Gen Comp Endocrinol 133:287–296
648. **Stoker C, Beldoménico PM, Bosquiazzo VL, Zayas MA, Rey F, Rodríguez H, Muñoz-de-Toro M, Luque EH** 2008 Developmental exposure to endocrine disruptor chemicals alters follicular dynamics and steroid levels in *Caiman latirostris*. Gen Comp Endocrinol 156:603–612
649. **Crain DA, Guillette Jr LJ, Rooney AA, Pickford DB** 1997 Alterations in steroidogenesis in alligators (*Alligator mississippiensis*) exposed naturally and experimentally to environmental contaminants. Environ Health Perspect 105:528–533
650. **Mukhi S, Patiño R** 2007 Effects of prolonged exposure to perchlorate on thyroid and reproductive function in zebrafish. Toxicol Sci 96:246–254
651. **Mukhi S, Torres L, Patiño R** 2007 Effects of larval-juvenile treatment with perchlorate and co-treatment with thyroxine on zebrafish sex ratios. Gen Comp Endocrinol 150:486–494
652. **Bernhardt RR, von Hippel FA, O'Hara TM** 2011 Chronic perchlorate exposure causes morphological abnormalities in developing stickleback. Environ Toxicol Chem 30:1468–1478
653. **Li W, Zha J, Yang L, Li Z, Wang Z** 2011 Regulation of iodothyronine deiodinases and sodium iodide symporter mRNA expression by perchlorate in larvae and adult Chinese rare minnow (*Gobiocypris rarus*). Marine Pollut Bull 63:350–355
654. **Goleman WL, Urquidi LJ, Anderson TA, Smith EE, Kendall RJ, Carr JA** 2002 Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in *Xenopus laevis*. Environ Toxicol Chem 21:424–430
655. **Ortiz-Santaliestra ME, Sparling DW** 2007 Alteration of larval development and metamorphosis by nitrate and perchlorate in southern leopard frogs (Rana sphenocephala). Arch Environ Contam Toxicol 53:639–646
656. **Hornung MW, Degitz SJ, Korte LM, Olson JM, Kosian PA, Linnum AL, Tietge JE** 2010 Inhibition of thyroid hormone release from cultured amphibian thyroid glands by methimazole, 6-propylthiouracil, and perchlorate. Toxicol Sci 118:42–51
657. **Opitz R, Kloas W** 2010 Developmental regulation of gene expression in the thyroid gland of *Xenopus laevis* tadpoles. Gen Comp Endocrinol 168:199–208
658. **Tietge JE, Butterworth BC, Haselman JT, Holcombe GW, Hornung MW, Korte JJ, Kosian PA, Wolfe M, Degitz SJ** 2010 Early temporal effects of three thyroid hormone synthesis inhibitors in *Xenopus laevis*. Aquat Toxicol 98:44–50
659. **Chen Y, Sible JC, McNabb FMA** 2008 Effects of maternal exposure to ammonium perchlorate on thyroid function and the expression of thyroid-responsive genes in Japanese quail embryos. Gen Comp Endocrinol 159:196–207
660. **Chen Y, McNabb FM, Sible JC** 2009 Perchlorate exposure induces hypothyroidism and affects thyroid-responsive genes in liver but not brain of quail chicks. Arch Environ Contam Toxicol 57:598–607
661. **Pflugfelder O** 1959 The alteration of the thyroid and other organs of the domestic fowl by potassium perchlorate, with comparative studies on lower vertebrates. Wilhelm Roux Arch Entwicklungsmech Organ 151:78–112
662. **Dent JN, Lynn WG** 1958 A comparison of the effects of goitrogens on thyroid activity in *Triturus viridescens* and *Desmognathus fuscus*. Biol Bull 115:411–420
663. **Fox GA** 2001 Wildlife as sentinels of human health effects

in the Great Lakes–St. Lawrence basin. Environ Health Perspect 109(Suppl 6):853–861
664. **Tanabe S** 2002 Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. Mar Pollut Bull 45:69–77
665. **Carney SA, Prasch AL, Heideman W, Peterson RE** 2006 Understanding dioxin developmental toxicity using the zebrafish model. Birth Defects Res A Clin Mol Teratol 76: 7–18
666. **Fisk AT, de Wit CA, Wayland M, Kuzyk ZZ, Burgess N, Letcher R, Braune B, Norstrom R, Blum SP, Sandau C, Lie E, Larsen HJ, Skaare JU, Muir DC** 2005 An assessment of the toxicological significance of anthropogenic contaminants in Canadian arctic wildlife. Sci Total Environ 351–352:57–93
667. **Cooper KR, Wintermyer M** 2009 A critical review: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) effects on gonad development in bivalve mollusks. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27:226–245
668. **Van den Berg M, Birnbaum L, Bosveld AT, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FX, Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T** 1998 Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 106:775–792
669. **Gray LE, Ostby J, Wolf C, Lambright C, Kelce W** 1998 The value of mechanistic studies in laboratory animals for the prediction of reproductive effects in wildlife: endocrine effects on mammalian sexual differentiation. Environ Toxicol Chem 17:109–118
670. **Hayes TB** 1998 Endocrine disruptors in amphibians: potential impacts and the usefulness of amphibian screens for detecting endocrine disrupting compounds. Sci J (Kagaku) 68:557–568
671. **Colborn T** 1994 The wildlife/human connection: modernizing risk decisions. Environ Health Perspect 102:55–59
672. **Colborn T** 1995 Environmental estrogens: health implications for humans and wildlife. Environ Health Perspect 103:135–136
673. **Harrison PT, Holmes P, Humfrey CD** 1997 Reproductive health in humans and wildlife: are adverse trends associated with environmental chemical exposure? Sci Total Environ 205:97–106
674. **Edwards TM, Moore BC, Guillette Jr LJ** 2006 Reproductive dysgenesis in wildlife: a comparative view. Int J Androl 29:109–121
675. **Rhind SM** 2009 Anthropogenic pollutants: a threat to ecosystem sustainability? Philos Trans R Soc Lond B Biol Sci 364:3391–3401
676. **Decensi A, Gandini S, Guerrieri-Gonzaga A, Johansson H, Manetti L, Bonanni B, Sandri MT, Barreca A, Costa A, Robertson C, Lien EA** 1999 Effect of blood tamoxifen concentrations on surrogate biomarkers in a trial of dose reduction in healthy women. J Clin Oncol 17:2633–2638
677. **Kisanga ER, Gjerde J, Guerrieri-Gonzaga A, Pigatto F, Pesci-Feltri A, Robertson C, Serrano D, Pelosi G, Decensi A, Lien EA** 2004 Tamoxifen and metabolite concentrations in serum and breast cancer tissue during three dose regimens in a randomized preoperative trial. Clin Cancer Res 10:2336–2343
678. **Nagel SC, vom Saal FS, Welshons WV** 1998 The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: physiology of delivery modifies estrogenic activity. Proc Soc Exp Biol Med 217:300–309
679. **Lakind JS, Naiman DQ** 2008 Bisphenol A (BPA) daily intakes in the United States: estimates from the 2003–2004 NHANES urinary BPA data. J Expo Sci Environ Epidemiol 18:608–615
680. **Wittassek M, Koch HM, Angerer J, Brüning T** 2011 Assessing exposure to phthalates: the human biomonitoring approach. Mol Nutr Food Res 55:7–31
681. **David RM, Moore MR, Finney DC, Guest D** 2000 Chronic toxicity of di(2-ethylhexyl)phthalate in rats. Toxicol Sci 55:433–443
682. **Agency for Toxic Substances and Diseases Registry** 2011 Toxic substances portal: di(2-ethylhexyl)phthalate (DEHP). Atlanta, GA: Centers for Disease Control
683. **Dickerson SM, Cunningham SL, Patisaul HB, Woller MJ, Gore AC** 2011 Endocrine disruption of brain sexual differentiation by developmental PCB exposure. Endocrinology 152:581–594
684. **Salama J, Chakraborty TR, Ng L, Gore AC** 2003 Effects of polychlorinated biphenyls on estrogen receptor-$\beta$ expression in the anteroventral periventricular nucleus. Environ Health Perspect 111:1278–1282
685. **Cassidy RA, Vorhees CV, Minnema DJ, Hastings L** 1994 The effects of chlordane exposure during pre- and postnatal periods at environmentally relevant levels on sex steroid-mediated behaviors and functions in the rat. Toxicol Appl Pharmacol 126:326–337
686. **McMahon T, Halstead N, Johnson S, Raffel TR, Romansic JM, Crumrine PW, Boughton RK, Martin LB, Rohr JR** 2011 The fungicide chlorothalonil is nonlinearly associated with corticosterone levels, immunity, and mortality in amphibians. Environ Health Perspect 119:1098–1103
687. **Guo-Ross SX, Chambers JE, Meek EC, Carr RL** 2007 Altered muscarinic acetylcholine receptor subtype binding in neonatal rat brain following exposure to chlorpyrifos or methyl parathion. Toxicol Sci 100:118–127
688. **Palanza P, Parmigiani S, Liu H, vom Saal FS** 1999 Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o,p'-DDT alters aggressive behavior of male and female house mice. Pharmacol Biochem Behav 64:665–672
689. **vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV** 1997 Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci USA 94:2056–2061
690. **Slikker Jr W, Scallet AC, Doerge DR, Ferguson SA** 2001 Gender-based differences in rats after chronic dietary exposure to genistein. Int J Toxicol 20:175–179
691. **Smialowicz RJ, Williams WC, Copeland CB, Harris MW, Overstreet D, Davis BJ, Chapin RE** 2001 The effects of perinatal/juvenile heptachlor exposure on adult immune and reproductive system function in rats. Toxicol Sci 61: 164–175

692. **Valkusz Z, Nagyéri G, Radács M, Ocskó T, Hausinger P, László M, László FA, Juhász A, Julesz J, Pálföldi R, Gálfi M** 2011 Further analysis of behavioral and endocrine consequences of chronic exposure of male Wistar rats to subtoxic doses of endocrine disruptor chlorobenzenes. Physiol Behav 103:421–430
693. **Manfo FP, Chao WF, Moundipa PF, Pugeat M, Wang PS** 2011 Effects of maneb on testosterone release in male rats. Drug Chem Toxicol 34:120–128
694. **Chapin RE, Harris MW, Davis BJ, Ward SM, Wilson RE, Mauney MA, Lockhart AC, Smialowicz RJ, Moser VC, Burka LT, Collins BJ** 1997 The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. Fundam Appl Toxicol 40: 138–157
695. **White Jr KL, Germolec DR, Booker CD, Hernendez DM, McCay JA, Delclos KB, Newbold RR, Weis C, Guo TL** 2005 Dietary methoxychlor exposure modulates splenic natural killer cell activity, antibody-forming cell response and phenotypic marker expression in F0 and F1 generations of Sprague Dawley rats. Toxicology 207:271–281
696. **Faass O, Schlumpf M, Reolon S, Henseler M, Maerkel K, Durrer S, Lichtensteiger W** 2009 Female sexual behavior, estrous cycle and gene expression in sexually dimorphic brain regions after pre- and postnatal exposure to endocrine active UV filters. Neurotoxicology 30:249–260
697. **Lemini C, Hernández A, Jaimez R, Franco Y, Avila ME, Castell A** 2004 Morphometric analysis of mice uteri treated with the preservatives methyl, ethyl, propyl, and butylparaben. Toxicol Ind Health 20:123–132
698. **Damgaard IN, Jensen TK, Petersen JH, Skakkebaek NE, Toppari J, Main KM** 2008 Risk factors for congenital cryptorchidism in a prospective birth cohort study. PLoS ONE 3:e3051
699. **Laurenzana EM, Weis CC, Bryant CW, Newbold R, Delclos KB** 2002 Effect of dietary administration of genistein, nonylphenol or ethinyl estradiol on hepatic testosterone metabolism, cytochrome P-450 enzymes, and estrogen receptor $\alpha$ expression. Food Chem Toxicol 40:53–63
700. **Tyl RW, Myers CB, Marr MC, Brine DR, Fail PA, Seely JC, Van Miller JP** 1999 Two-generation reproduction study with para-tert-octylphenol in rats. Regul Toxicol Pharmacol 30:81–95
701. **Li E, Guo Y, Ning Q, Zhang S, Li D** 2011 Research for the effect of octylphenol on spermatogenesis and proteomic analysis in octylphenol-treated mice testes. Cell Biol Int 35:305–309
702. **Timofeeva OA, Sanders D, Seemann K, Yang L, Hermanson D, Regenbogen S, Agoos S, Kallepalli A, Rastogi A, Braddy D, Wells C, Perraut C, Seidler FJ, Slotkin TA, Levin ED** 2008 Persistent behavioral alterations in rats neonatally exposed to low doses of the organophosphate pesticide, parathion. Brain Res Bull 77:404–411
703. **Kuriyama SN, Wanner A, Fidalgo-Neto AA, Talsness CE, Koerner W, Chahoud I** 2007 Developmental exposure to low-dose PBDE-99: tissue distribution and thyroid hormone levels. Toxicology 242:80–90
704. **Tanaka T, Morita A, Kato M, Hirai T, Mizoue T, Terauchi Y, Watanabe S, Noda M** 2011 Congener-specific polychlorinated biphenyls and the prevalence of diabetes in the Saku Control Obesity Program (SCOP). Endocr J 58:589–596
705. **Buckman AH, Fisk AT, Parrott JL, Solomon KR, Brown SB** 2007 PCBs can diminish the influence of temperature on thyroid indices in rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol 84:366–378
706. **Jiang Y, Zhao J, Van Audekercke R, Dequeker J, Geusens P** 1996 Effects of low-dose long-term sodium fluoride preventive treatment on rat bone mass and biomechanical properties. Calcif Tissue Int 58:30–39
707. **Kirchner S, Kieu T, Chow C, Casey S, Blumberg B** 2010 Prenatal exposure to the environmental obesogen tributyltin predisposes multipotent stem cells to become adipocytes. Mol Endocrinol 24:526–539
708. **Stoker TE, Gibson EK, Zorrilla LM** 2010 Triclosan exposure modulates estrogen-dependent responses in the female wistar rat. Toxicol Sci 117:45–53
709. **Eustache F, Mondon F, Canivenc-Lavier MC, Lesaffre C, Fulla Y, Berges R, Cravedi JP, Vaiman D, Auger J** 2009 Chronic dietary exposure to a low-dose mixture of genistein and vinclozolin modifies the reproductive axis, testis transcriptome, and fertility. Environ Health Perspect 117:1272–1279
710. **Schlumpf M, Durrer S, Faass O, Ehnes C, Fuetsch M, Gaille C, Henseler M, Hofkamp L, Maerkel K, Reolon S, Timms B, Tresguerres JA, Lichtensteiger W** 2008 Developmental toxicity of UV filters and environmental exposure: a review. Int J Androl 31:144–151
711. **Schlecht C, Klammer H, Wuttke W, Jarry H** 2006 A dose-response study on the estrogenic activity of benzophenone-2 on various endpoints in the serum, pituitary and uterus of female rats. Arch Toxicol 80:656–661
712. **Sitarek K** 2001 Embryolethal and teratogenic effects of carbendazim in rats. Teratog Carcinog Mutagen 21:335–340
713. **Higashihara N, Shiraishi K, Miyata K, Oshima Y, Minobe Y, Yamasaki K** 2007 Subacute oral toxicity study of bisphenol F based on the draft protocol for the "Enhanced OECD Test Guideline no. 407". Arch Toxicol 81:825–832
714. **Yamano Y, Ohyama K, Ohta M, Sano T, Ritani A, Shimada J, Ashida N, Yoshida E, Ikehara K, Morishima I** 2005 A novel spermatogenesis related factor-2 (SRF-2) gene expression affected by TCDD treatment. Endocr J 52:75–81
715. **Ikeda M, Tamura M, Yamashita J, Suzuki C, Tomita T** 2005 Repeated *in utero* and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure affects male gonads in offspring, leading to sex ratio changes in F2 progeny. Toxicol Appl Pharmacol 206:351–355
716. **Welshons WV, Nagel SC, Thayer KA, Judy BM, Vom Saal FS** 1999 Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol Ind Health 15:12–25
717. **Christian M, Gillies G** 1999 Developing hypothalamic dopaminergic neurones as potential targets for environmental estrogens. J Endocrinol 160:R1–R6
718. **Jeng YJ, Watson CS** 2011 Combinations of physiologic estrogens with xenoestrogens alter ERK phosphorylation

profiles in rat pituitary cells. Environ Health Perspect 119: 104–112
719. **Jeng YJ, Kochukov MY, Watson CS** 2009 Membrane estrogen receptor-$\alpha$-mediated nongenomic actions of phytoestrogens in GH3/B6/F10 pituitary tumor cells. J Mol Signal 4:2
720. **Narita S, Goldblum RM, Watson CS, Brooks EG, Estes DM, Curran EM, Midoro-Horiuti T** 2007 Environmental estrogens induce mast cell degradulation and enhance IgE-mediated release of allergic mediators. Environ Health Perspect 115:48–52
721. **Somjen D, Kohen F, Jaffe A, Amir-Zaltsman Y, Knoll E, Stern N** 1998 Effects of gonadal steroids and their antagonists on DNA synthesis in human vascular cells. Hypertension 32:39–45
722. **Devidze N, Fujimori K, Urade Y, Pfaff DW, Mong JA** 2010 Estradiol regulation of lipocalin-type prostaglandin D synthase promoter activity: evidence for direct and indirect mechanisms. Neurosci Lett 474:17–21
723. **Du J, Wang Y, Hunter R, Wei Y, Blumenthal R, Falke C, Khairova R, Zhou R, Yuan P, Machado-Vieira R, McEwen BS, Manji HK** 2009 Dynamic regulation of mitochondrial function by glucocorticoids. Proc Natl Acad Sci USA 106: 3543–3548
724. **Guillen C, Bartolomé A, Nevado C, Benito M** 2008 Biphasic effect of insulin on $\beta$ cell apoptosis depending on glucose deprivation. FEBS Lett 582:3855–3860
725. **Welsh Jr TH, Kasson BG, Hsueh AJ** 1986 Direct biphasic modulation of gonadotropin-stimulated testicular androgen biosynthesis by prolactin. Biol Reprod 34:796–804
726. **Sarkar PK** 2008 L-Triiodothyronine differentially and nongenomically regulates synaptosomal protein phosphorylation in adult rat brain cerebral cortex: role of calcium and calmodulin. Life Sci 82:920–927
727. **Calvo RM, Obregon MJ** 2009 Tri-iodothyronine upregulates adipnutrin mRNA expression in rat and human adipocytes. Mol Cell Endocrinol 311:39–46
728. **Leung LY, Kwong AK, Man AK, Woo NY** 2008 Direct actions of cortisol, thyroxine and growth hormone on IGF-I mRNA expression in sea bream hepatocytes. Comp Biochem Physiol A Mol Integr Physiol 151:705–710
729. **Habauzit D, Boudot A, Kerdivel G, Flouriot G, Pakdel F** 2010 Development and validation of a test for environmental estrogens: checking xeno-estrogen activity by CXCL12 secretion in breast cancer cell lines (CXCL-test). Environ Toxicol 25:495–503
730. **Boettcher M, Kosmehl T, Braunbeck T** 2011 Low-dose effects and biphasic effect profiles: Is trenbolone a genotoxicant? Mutat Res 723:152–157
731. **Wetherill YB, Petre CE, Monk KR, Puga A, Knudsen KE** 2002 The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostatic adenocarcinoma cells. Mol Cancer Ther 1:515–524
732. **Sandy EH, Yao J, Zheng S, Gogra AB, Chen H, Zheng H, Yormah TB, Zhang X, Zaray G, Ceccanti B, Choi MM** 2010 A comparative cytotoxicity study of isomeric alkylphthalates to metabolically variant bacteria. J Hazard Mater 182:631–639
733. **Murono EP, Derk RC, de León JH** 1999 Biphasic effects of octylphenol on testosterone biosynthesis by cultured Leydig cells from neonatal rats. Reprod Toxicol 13:451–462
734. **Benísek M, Bláha L, Hilscherová K** 2008 Interference of PAHs and their N-heterocyclic analogs with signaling of retinoids in vitro. Toxicol In Vitro 22:1909–1917
735. **Beníšek M, Kubincová P, Bláha L, Hilscherová K** 2011 The effects of PAHs and N-PAHs on retinoid signaling and Oct-4 expression in vitro. Toxicol Lett 200:169–175
736. **Evanson M, Van Der Kraak GJ** 2001 Stimulatory effects of selected PAHs on testosterone production in goldfish and rainbow trout and possible mechanisms of action. Comp Biochem Physiol C Toxicol Pharmacol 130:249–258
737. **Chaube R, Mishra S, Singh RK** 2010 In vitro effects of lead nitrate on steroid profiles in the post-vitellogenic ovary of the catfish Heteropneustes fossilis. Toxicol In Vitro 24: 1899–1904
738. **Helmestam M, Stavreus-Evers A, Olovsson M** 2010 Cadmium chloride alters mRNA levels of angiogenesis related genes in primary human endometrial endothelial cells grown in vitro. Reprod Toxicol 30:370–376
739. **Chen AC, Donovan SM** 2004 Genistein at a concentration present in soy infant formula inhibits Caco-2BBe cell proliferation by causing G2/M cell cycle arrest. J Nutr 134: 1303–1308
740. **El Touny LH, Banerjee PP** 2009 Identification of a biphasic role for genistein in the regulation of prostate cancer growth and metastasis. Cancer Res 69:3695–3703
741. **Guo JM, Xiao BX, Liu DH, Grant M, Zhang S, Lai YF, Guo YB, Liu Q** 2004 Biphasic effect of daidzein on cell growth of human colon cancer cells. Food Chem Toxicol 42:1641–1646
742. **Wang H, Zhou H, Zou Y, Liu Q, Guo C, Gao G, Shao C, Gong Y** 2010 Resveratrol modulates angiogenesis through the GSK3$\beta$/$\beta$-catenin/TCF-dependent pathway in human endothelial cells. Biochem Pharmacol 80:1386–1395
743. **Pedro M, Lourenço CF, Cidade H, Kijjoa A, Pinto M, Nascimento MS** 2006 Effects of natural prenylated flavones in the phenotypical ER (+) MCF-7 and ER (−) MDA-MB-231 human breast cancer cells. Toxicol Lett 164:24–36
744. **Almstrup K, Fernández MF, Petersen JH, Olea N, Skakkebaek NE, Leffers H** 2002 Dual effects of phytoestrogens result in U-shaped dose-response curves. Environ Health Perspect 110:743–748
745. **Pinto B, Bertoli A, Noccioli C, Garritano S, Reali D, Pistelli L** 2008 Estradiol-antagonistic activity of phenolic compounds from leguminous plants. Phytother Res 22:362–366
746. **Sanderson JT, Hordijk J, Denison MS, Springsteel MF, Nantz MH, van den Berg M** 2004 Induction and inhibition of aromatase (CYP19) activity by natural and synthetic flavonoid compounds in H295R human adrenocortical carcinoma cells. Toxicol Sci 82:70–79
747. **Elattar TM, Virji AS** 2000 The inhibitory effect of curcumin, genistein, quercetin and cisplatin on the growth of oral cancer cells in vitro. Anticancer Res 20:1733–1738
748. **Ahn NS, Hu H, Park JS, Park JS, Kim JS, An S, Kong G, Aruoma OI, Lee YS, Kang KS** 2005 Molecular mechanisms of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced inverted U-shaped dose responsiveness in anchorage independent growth and cell proliferation of human breast epithelial cells with stem cell characteristics. Mutat Res 579: 189–199
749. **Dickerson SM, Guevara E, Woller MJ, Gore AC** 2009 Cell

death mechanisms in GT1–7 GnRH cells exposed to polychlorinated biphenyls PCB74, PCB118, PCB153. Toxicol Appl Pharmacol 237:237–245

750. **Campagna C, Ayotte P, Sirard MA, Arsenault G, Laforest JP, Bailey JL** 2007 Effect of an environmentally relevant metabolized organochlorine mixture on porcine cumulus-oocyte complexes. Reprod Toxicol 23:145–152
751. **Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE** 2009 Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262:184–191
752. **Greenman SB, Rutten MJ, Fowler WM, Scheffler L, Shortridge LA, Brown B, Sheppard BC, Deveney KE, Deveney CW, Trunkey DD** 1997 Herbicide/pesticide effects on intestinal epithelial growth. Environ Res 75:85–93
753. **Sreeramulu K, Liu R, Sharom FJ** 2007 Interaction of insecticides with mammalian P-glycoprotein and their effect on its transport function. Biochim Biophys Acta 1768: 1750–1757
754. **Asp V, Ullerås E, Lindström V, Bergström U, Oskarsson A, Brandt I** 2010 Biphasic hormonal responses to the adrenocorticolytic DDT metabolite 3-methylsulfonyl-DDE in human cells. Toxicol Appl Pharmacol 242:281–289
755. **Ralph JL, Orgebin-Crist MC, Lareyre JJ, Nelson CC** 2003 Disruption of androgen regulation in the prostate by the environmental contaminant hexachlorobenzene. Environ Health Perspect 111:461–466
756. **Ohlsson A, Ullerås E, Oskarsson A** 2009 A biphasic effect of the fungicide prochloraz on aldosterone, but not cortisol, secretion in human adrenal H295R cells: underlying mechanisms. Toxicol Lett 191:174–180
757. **Ohlsson A, Cedergreen N, Oskarsson A, Ullerås E** 2010 Mixture effects of imidazole fungicides on cortisol and aldosterone secretion in human adrenocortical H295R cells. Toxicology 275:21–28
758. **Kim KH, Bose DD, Ghogha A, Riehl J, Zhang R, Barnhart CD, Lein PJ, Pessah IN** 2011 Para- and ortho-substitutions are key determinants of polybrominated diphenyl ether activity toward ryanodine receptors and neurotoxicity. Environ Health Perspect 119:519–526
759. **Alm H, Scholz B, Kultima K, Nilsson A, Andrén PE, Savitski MM, Bergman A, Stigson M, Fex-Svenningsen A, Dencker L** 2010 In vitro neurotoxicity of PBDE-99: immediate and concentration-dependent effects on protein expression in cerebral cortex cells. J Proteome Res 9:1226–1235
760. **Sànchez JJ, Abreu P, González-Hernández T, Hernández A, Prieto L, Alonso R** 2004 Estrogen modulation of adrenoceptor responsiveness in the female rat pineal gland: differential expression of intracellular estrogen receptors. J Pineal Res 37:26–35
761. **Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL** 1996 Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environ Health Perspect 104:1296–1300
762. **Dhir A, Kulkarni SK** 2008 Antidepressant-like effect of 17$\beta$-estradiol: involvement of dopaminergic, serotonergic, and (or) sigma-1 receptor systems. Can J Physiol Pharmacol 86:726–735
763. **Ribeiro AC, Pfaff DW, Devidze N** 2009 Estradiol modulates behavioral arousal and induces changes in gene expression profiles in brain regions involved in the control of vigilance. Eur J Neurosci 29:795–801
764. **Park CR, Campbell AM, Woodson JC, Smith TP, Fleshner M, Diamond DM** 2006 Permissive influence of stress in the expression of a U-shaped relationship between serum corticosterone levels and spatial memory errors in rats. Dose Response 4:55–74
765. **Abrahám I, Harkany T, Horvath KM, Veenema AH, Penke B, Nyakas C, Luiten PG** 2000 Chronic corticosterone administration dose-dependently modulates A$\beta$(1–42)- and NMDA-induced neurodegeneration in rat magnocellular nucleus basalis. J Neuroendocrinol 12: 486–494
766. **Duclos M, Gouarne C, Martin C, Rocher C, Mormède P, Letellier T** 2004 Effects of corticosterone on muscle mitochondria identifying different sensitivity to glucocorticoids in Lewis and Fischer rats. Am J Physiol Endocrinol Metab 286:E159–E167
767. **Abrari K, Rashidy-Pour A, Semnanian S, Fathollahi Y, Jadid M** 2009 Post-training administration of corticosterone enhances consolidation of contextual fear memory and hippocampal long-term potentiation in rats. Neurobiol Learn Mem 91:260–265
768. **Spée M, Marchal L, Thierry AM, Chastel O, Enstipp M, Maho YL, Beaulieu M, Raclot T** 2011 Exogenous corticosterone mimics a late fasting stage in captive Adelie penguins (*Pygoscelis adeliae*). Am J Physiol Regul Integr Comp Physiol 300:R1241–R1249
769. **Sunny F, Oommen VO** 2004 Effects of steroid hormones on total brain $Na^+$-$K^+$ ATPase activity in *Oreochromis mossambicus*. Indian J Exp Biol 42:283–287
770. **Huggard D, Khakoo Z, Kassam G, Mahmoud SS, Habibi HR** 1996 Effect of testosterone on maturational gonadotropin subunit messenger ribonucleic acid levels in the goldfish pituitary. Biol Reprod 54:1184–1191
771. **Ren SG, Huang Z, Sweet DE, Malozowski S, Cassorla F** 1990 Biphasic response of rat tibial growth to thyroxine administration. Acta Endocrinol (Copenh) 122:336–340
772. **Houshmand F, Faghihi M, Zahediasl S** 2009 Biphasic protective effect of oxytocin on cardiac ischemia/reperfusion injury in anaesthetized rats. Peptides 30:2301–2308
773. **Boccia MM, Kopf SR, Baratti CM** 1998 Effects of a single administration of oxytocin or vasopressin and their interactions with two selective receptor antagonists on memory storage in mice. Neurobiol Learn Mem 69:136–146
774. **Tai SH, Hung YC, Lee EJ, Lee AC, Chen TY, Shen CC, Chen HY, Lee MY, Huang SY, Wu TS** 2011 Melatonin protects against transient focal cerebral ischemia in both reproductively active and estrogen-deficient female rats: the impact of circulating estrogen on its hormetic dose-response. J Pineal Res 50:292–303
775. **Cai JX, Arnsten AF** 1997 Dose-dependent effects of the dopamine D1 receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. J Pharmacol Exp Ther 283:183–189
776. **Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF** 2007 Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nat Neurosci 10:376–384
777. **Palanza P, Parmigiani S, vom Saal FS** 2001 Effects of prenatal exposure to low doses of diethylstilbestrol, o,p'DDT,

and methoxychlor on postnatal growth and neurobehavioral development in male and female mice. Horm Behav 40:252–265

778. **Thuillier R, Wang Y, Culty M** 2003 Prenatal exposure to estrogenic compounds alters the expression pattern of platelet-derived growth factor receptors $\alpha$ and $\beta$ in neonatal rat testis: identification of gonocytes as targets of estrogen exposure. Biol Reprod 68:867–880
779. **Köhlerová E, Skarda J** 2004 Mouse bioassay to assess oestrogenic and anti-oestrogenic compounds: hydroxytamoxifen, diethylstilbestrol and genistein. J Vet Med A Physiol Pathol Clin Med 51:209–217
780. **Putz O, Schwartz CB, Kim S, LeBlanc GA, Cooper RL, Prins GS** 2001 Neonatal low- and high-dose exposure to estradiol benzoate in the male rat. I. Effects on the prostate gland. Biol Reprod 65:1496–1505
781. **Rochester JR, Forstmeier W, Millam JR** 2010 Post-hatch oral estrogen in zebra finches (*Taeniopygia guttata*): is infertility due to disrupted testes morphology or reduced copulatory behavior? Physiol Behav 101:13–21
782. **Vosges M, Le Page Y, Chung BC, Combarnous Y, Porcher JM, Kah O, Brion F** 2010 17$\alpha$-Ethinylestradiol disrupts the ontogeny of the forebrain GnRH system and the expression of brain aromatase during early development of zebrafish. Aquat Toxicol 99:479–491
783. **Gust M, Buronfosse T, Giamberini L, Ramil M, Mons R, Garric J** 2009 Effects of fluoxetine on the reproduction of two prosobranch mollusks: *Potamopyrgus antipodarum* and *Valvata piscinalis*. Environ Pollut 157:423–429
784. **Villeneuve DL, Knoebl I, Kahl MD, Jensen KM, Hammermeister DE, Greene KJ, Blake LS, Ankley GT** 2006 Relationship between brain and ovary aromatase activity and isoform-specific aromatase mRNA expression in the fathead minnow (*Pimephales promelas*). Aquat Toxicol 76: 353–368
785. **Jones BA, Shimell JJ, Watson NV** 2011 Pre- and postnatal Bisphenol A treatment results in persistent deficits in the sexual behavior of male rats, but not female rats, in adulthood. Horm Behav 59:246–251
786. **Lemos MF, Esteves AC, Samyn B, Timperman I, van Beeumen J, Correia A, van Gestel CA, Soares AM** 2010 Protein differential expression induced by endocrine disrupting compounds in a terrestrial isopod. Chemosphere 79:570–576
787. **Nishizawa H, Morita M, Sugimoto M, Imanishi S, Manabe N** 2005 Effects of *in utero* exposure to bisphenol A on mRNA expression of arylhydrocarbon and retinoid receptors in murine embryos. J Reprod Dev 51:315–324
788. **Andrade AJ, Grande SW, Talsness CE, Grote K, Chahoud I** 2006 A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl)-phthalate (DEPH): non-monotonic dose-response and low dose effects on rat brain aromatase activity. Toxicology 227:185–192
789. **Ge RS, Chen GR, Dong Q, Akingbemi B, Sottas CM, Santos M, Sealfon SC, Bernard DJ, Hardy MP** 2007 Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. J Androl 28:513–520
790. **Grande SW, Andrade AJ, Talsness CE, Grote K, Chahoud I** 2006 A dose-response study following *in utero* and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicol Sci 91:247–254
791. **Vo TT, Jung EM, Dang VH, Yoo YM, Choi KC, Yu FH, Jeung EB** 2009 Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. Reprod Biol Endocrinol 7:104
792. **Takano H, Yanagisawa R, Inoue K, Ichinose T, Sadakane K, Yoshikawa T** 2006 Di-(2-ethylhexyl) phthalate enhances atopic dermatitis-like skin lesions in mice. Environ Health Perspect 114:1266–1269
793. **Oliveira-Filho EC, Grisolia CK, Paumgartten FJR** 2009 Trans-generation study of the effects of nonylphenol ethoxylate on the reproduction of the snail *Biomphalaria tenagophila*. Ecotoxicol Environ Saf 72:458–465
794. **Duft M, Schulte-Oehlmann U, Weltje L, Tillmann M, Oehlmann J** 2003 Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail *Potamopyrgus antipodarum*. Aquat Toxicol 64:437–449
795. **Oehlmann J, Schulte-Oehlmann U, Tillmann M, Markert B** 2000 Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I. bisphenol A and octylphenol as xeno-estrogens. Ecotoxicology 9:383–397
796. **Maranghi F, Tassinari R, Marcoccia D, Altieri I, Catone T, De Angelis G, Testai E, Mastrangelo S, Evandri MG, Bolle P, Lorenzetti S** 2010 The food contaminant semicarbazide acts as an endocrine disrupter: evidence from an integrated in vivo/in vitro approach. Chem Biol Interact 183:40–48
797. **Giudice BD, Young TM** 2010 The antimicrobial triclocarban stimulates embryo production in the freshwater mudsnail *Potamopyrgus antipodarum*. Environ Toxicol Chem 29:966–970
798. **Love OP, Shutt LJ, Silfies JS, Bortolotti GR, Smits JE, Bird DM** 2003 Effects of dietary PCB exposure on adrenocortical function in captive American kestrels (*Falco sparverius*). Ecotoxicology 12:199–208
799. **Franceschini MD, Custer CM, Custer TW, Reed JM, Romero LM** 2008 Corticosterone stress response in tree swallows nesting near polychlorinated biphenyl- and dioxin-contaminated rivers. Environ Toxicol Chem 27: 2326–2331
800. **Axelstad M, Boberg J, Hougaard KS, Christiansen S, Jacobsen PR, Mandrup KR, Nellemann C, Lund SP, Hass U** 2011 Effects of pre- and postnatal exposure to the UV-filter octyl methoxycinnamate (OMC) on the reproductive, auditory and neurological development of rat offspring. Toxicol Appl Pharmacol 250:278–290
801. **Riegel AC, French ED** 1999 Acute toluene induces biphasic changes in rat spontaneous locomotor activity which are blocked by remoxipride. Pharmacol Biochem Behav 62: 399–402
802. **Fan F, Wierda D, Rozman KK** 1996 Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on humoral and cell-mediated immunity in Sprague-Dawley rats. Toxicology 106: 221–228
803. **Teeguarden JG, Dragan YP, Singh J, Vaughan J, Xu YH, Goldsworthy T, Pitot HC** 1999 Quantitative analysis of dose- and time-dependent promotion of four phenotypes of altered hepatic foci by 2,3,7,8-tetrachlorodibenzo-*p*-di-

oxin in female Sprague-Dawley rats. Toxicol Sci 51:211–223
804. **Höfer N, Diel P, Wittsiepe J, Wilhelm M, Kluxen FM, Degen GH** 2010 Investigations on the estrogenic activity of the metallohormone cadmium in the rat intestine. Arch Toxicol 84:541–552
805. **Zhang Y, Shen G, Yu Y, Zhu H** 2009 The hormetic effect of cadmium on the activity of antioxidant enzymes in the earthworm *Eisenia fetida*. Environ Pollut 157:3064–3068
806. **Sharma B, Patiño R** 2009 Effects of cadmium on growth, metamorphosis and gonadal sex differentiation in tadpoles of the African clawed frog, *Xenopus laevis*. Chemosphere 76:1048–1055
807. **Wang CR, Tian Y, Wang XR, Yu HX, Lu XW, Wang C, Wang H** 2010 Hormesis effects and implicative application in assessment of lead-contaminated soils in roots of *Vicia faba* seedlings. Chemosphere 80:965–971
808. **Fox DA, Kala SV, Hamilton WR, Johnson JE, O'Callaghan JP** 2008 Low-level human equivalent gestational lead exposure produces supernormal scotopic electroretinograms, increased retinal neurogenesis, and decreased retinal dopamine utilization in rats. Environ Health Perspect 116:618–625
809. **Chiang EC, Shen S, Kengeri SS, Xu H, Combs GF, Morris JS, Bostwick DG, Waters DJ** 2009 Defining the optimal selenium dose for prostate cancer risk reduction: insights from the U-shaped relationship between selenium status, DNA damage, and apoptosis. Dose Response 8:285–300
810. **Harding LE** 2008 Non-linear uptake and hormesis effects of selenium in red-winged blackbirds (*Agelaius phoeniceus*). Sci Total Environ 389:350–366
811. **Wisniewski AB, Cernetich A, Gearhart JP, Klein SL** 2005 Perinatal exposure to genistein alters reproductive development and aggressive behavior in male mice. Physiol Behav 84:327–334
812. **Anderson JJ, Ambrose WW, Garner SC** 1998 Biphasic effects of genistein on bone tissue in the ovariectomized, lactating rat model. Proc Soc Exp Biol Med 217:345–350
813. **Dey A, Guha P, Chattopadhyay S, Bandyopadhyay SK** 2009 Biphasic activity of resveratrol on indomethacin-induced gastric ulcers. Biochem Biophys Res Commun 381: 90–95
814. **Boccia MM, Kopf SR, Baratti CM** 1999 Phlorizin, a competitive inhibitor of glucose transport, facilitates memory storage in mice. Neurobiol Learn Mem 71:104–112
815. **Brodeur JC, Svartz G, Perez-Coll CS, Marino DJ, Herkovits J** 2009 Comparative susceptibility to atrazine of three developmental stages of *Rhinella arenarum* and influence on metamorphosis: non-monotonous acceleration of the time to climax and delayed tail resorption. Aquat Toxicol 91:161–170
816. **Freeman JL, Beccue N, Rayburn AL** 2005 Differential metamorphosis alters the endocrine response in anuran larvae exposed to T3 and atrazine. Aquat Toxicol 75:263–276
817. **Undeðer U, Schlumpf M, Lichtensteiger W** 2010 Effect of the herbicide pendimethalin on rat uterine weight and gene expression and in silico receptor binding analysis. Food Chem Toxicol 48:502–508
818. **Cavieres MF, Jaeger J, Porter W** 2002 Developmental toxicity of a commercial herbicide mixture in mice. I. Effects on embryo implantation and litter size. Environ Health Perspect 110:1081–1085
819. **Zorrilla LM, Gibson EK, Stoker TE** 2010 The effects of simazine, a chlorotriazine herbicide, on pubertal development in the female Wistar rat. Reprod Toxicol 29:393–400
820. **Bloomquist JR, Barlow RL, Gillette JS, Li W, Kirby ML** 2002 Selective effects of insecticides on nigrostriatal dopaminergic nerve pathways. Neurotoxicology 23:537–544
821. **Lassiter TL, Brimijoin S** 2008 Rats gain excess weight after developmental exposure to the organophosphorothionate pesticide, chlorpyrifos. Neurotoxicol Teratol 30:125–130
822. **Wu H, Zhang R, Liu J, Guo Y, Ma E** 2011 Effects of malathion and chlorpyrifos on acetylcholinesterase and antioxidant defense system in *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae). Chemosphere 83:599–604
823. **Muthuviveganandavel V, Muthuraman P, Muthu S, Srikumar K** 2008 Toxic effects of carbendazim at low dose levels in male rats. J Toxicol Sci 33:25–30
824. **Laughlin GA, Goodell V, Barrett-Connor E** 2010 Extremes of endogenous testosterone are associated with increased risk of incident coronary events in older women. J Clin Endocrinol Metab 95:740–747
825. **Kratzik CW, Schatzl G, Lackner JE, Lunglmayr G, Brandstätter N, Rücklinger E, Huber J** 2007 Mood changes, body mass index and bioavailable testosterone in healthy men: results of the Androx Vienna Municipality Study. BJU Int 100:614–618
826. **Floege J, Kim J, Ireland E, Chazot C, Drueke T, de Francisco A, Kronenberg F, Marcelli D, Passlick-Deetjen J, Schernthaner G, Fouqueray B, Wheeler DC** 2010 Serum iPTH, calcium and phosphate, and the risk of mortality in a European haemodialysis population. Nephrol Dial Transplant 26:1948–1955
827. **Danese MD, Kim J, Doan QV, Dylan M, Griffiths R, Chertow GM** 2006 PTH and the risks for hip, vertebral, and pelvic fractures among patients on dialysis. Am J Kidney Dis 47:149–156
828. **Tan ZS, Beiser A, Vasan RS, Au R, Auerbach S, Kiel DP, Wolf PA, Seshadri S** 2008 Thyroid function and the risk of Alzheimer disease: the Framingham Study. Arch Intern Med 168:1514–1520
829. **Tanaka M, Fukui M, Tomiyasu K, Akabame S, Nakano K, Hasegawa G, Oda Y, Nakamura N** 2010 U-shaped relationship between insulin level and coronary artery calcification (CAC). J Atheroscler Thromb 17:1033–1040
830. **Pyörälä M, Miettinen H, Laakso M, Pyörälä K** 2000 Plasma insulin and all-cause, cardiovascular, and noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study. Diabetes Care 23:1097–1102
831. **Kumari M, Chandola T, Brunner E, Kivimaki M** 2010 A nonlinear relationship of generalized and central obesity with diurnal cortisol secretion in the Whitehall II study. J Clin Endocrinol Metab 95:4415–4423
832. **Bremmer MA, Deeg DJ, Beekman AT, Penninx BW, Lips P, Hoogendijk WJ** 2007 Major depression in late life is associated with both hypo- and hypercortisolemia. Biol Psychiatry 62:479–486
833. **Lee DH, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs Jr DR** 2010 Low dose of some persistent organic

pollutants predicts type 2 diabetes: a nested case-control study. Environ Health Perspect 118:1235–1242

834. **Mendez MA, Garcia-Esteban R, Guxens M, Vrijheid M, Kogevinas M, Goñi F, Fochs S, Sunyer J** 2011 Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ Health Perspect 119: 272–278
835. **Cho MR, Shin JY, Hwang JH, Jacobs DR Jr, Kim SY, Lee DH** 2011 Associations of fat mass and lean mass with bone mineral density differ by levels of persistent organic pollutants: National Health and Nutrition Examination Survey 1999–2004. Chemosphere 82:1268–1276
836. **Monica Lind P, Lind L** 10 May 2011 Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly. Atherosclerosis 10.1016/j.atherosclerosis.2011.1005.1001
837. **Melzer D, Rice N, Depledge MH, Henley WE, Galloway TS** 2010 Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. Environ Health Perspect 118:686–692
838. **Trabert B, De Roos AJ, Schwartz SM, Peters U, Scholes D, Barr DB, Holt VL** 2010 Non-dioxin-like polychlorinated biphenyls and risk of endometriosis. Environ Health Perspect 118:1280–1285
839. **Kim KY, Kim DS, Lee SK, Lee IK, Kang JH, Chang YS, Jacobs DR, Steffes M, Lee DH** 2010 Association of low-dose exposure to persistent organic pollutants with global DNA hypomethylation in healthy Koreans. Environ Health Perspect 118:370–374
840. **Laclaustra M, Navas-Acien A, Stranges S, Ordovas JM, Guallar E** 2009 Serum selenium concentrations and diabetes in U.S. adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. Atherosclerosis 117:1409–1413
841. **Laclaustra M, Stranges S, Navas-Acien A, Ordovas JM, Guallar E** 2010 Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. Atherosclerosis 210:643–648
842. **Ahmed S, Mahabbat-e Khoda S, Rekha RS, Gardner RM, Ameer SS, Moore S, Ekström EC, Vahter M, Raqib R** 2011 Arsenic-associated oxidative stress, inflammation, and immune disruption in human placenta and cord blood. Environ Health Perspect 119:258–264
843. **Claus Henn B, Ettinger AS, Schwartz J, Téllez-Rojo MM, Lamadrid-Figueroa H, Hernández-Avila M, Schnaas L, Amarasiriwardena C, Bellinger DC, Hu H, Wright RO** 2010 Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology 21:433–439
844. **Wirth JJ, Rossano MG, Daly DC, Paneth N, Puscheck E, Potter RC, Diamond MP** 2007 Ambient manganese exposure is negatively associated with human sperm motility and concentration. Epidemiology 18:270–273
845. **Lee DH, Lee IK, Porta M, Steffes M, Jacobs Jr DR** 2007 Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002. Diabetologia 50:1841–1851

# CHAPTER IV

# REGULATORY PROGRAMS AND RECOMMENDED INDUSTRY PROCEDURES

## INTRODUCTION

This chapter identifies and reviews the current State and Federal regulatory programs and industry-recommended procedures applicable to either reduce the potential for routine emissions and/or accidental hydrogen sulfide releases from oil and gas production or to mitigate the consequences of such emissions and releases.

## STATE REGULATIONS

Currently, there are no national ambient air quality standards (NAAQS) for $H_2S$. Most oil- and gas-producing States have their own regulations pertaining to $H_2S$ gas. Table IV-1 lists States that have set ambient air quality standards for $H_2S$ emissions.

The EPA gathered and reviewed several States' regulations and related guidance documents and later contacted State agencies to obtain additional information on the unique aspects of the State regulations governing $H_2S$ emissions in the oil and gas industry. EPA staff also met with officials from North Dakota during a trip to North Dakota oil and gas well sites. In addition, the Interstate Oil and Gas Compact Commission (IOGCC) was contacted to obtain information pertaining to regulatory programs (IOGCC, 1990).

This chapter contains a review of existing State regulations for nine States (California, Louisiana, Michigan, New Mexico, North Dakota, Oklahoma, Pennsylvania, Texas, and Wyoming).

These nine States were chosen for review because of their large production volumes, the potential presence of $H_2S$ in their well fields, and their distribution across the United States. The nine States contain over 68 percent of the total oil wells (419,989 wells/613,810 total U.S. wells) and 54 percent of the gas wells (147,360 wells/272,541 total U.S. wells) producing in the United States in 1991 (Petroleum Independent, 1992). For these States, regulatory agencies are identified, $H_2S$ regulations for routine emissions and accidental releases are described, enforcement programs are discussed, records and programs to track accidental $H_2S$ release are included, and the effectiveness of each State program is assessed qualitatively. The qualitative evaluation identifies existing control standards and the populations or ecosystems the standard is intended to protect.

In addition, these States account for 67 percent of the total U.S. oil production and 87 percent of the total U.S. natural gas production (Petroleum Independent, 1992). State regulations for $H_2S$ emissions from the oil and gas industry in Oklahoma, Texas, Michigan,

**Table IV-1. Ambient Air Quality Standards for $H_2S$**

| State | Concentration (ppb) | Average Time (hours) |
|---|---|---|
| California | 30 | 1 |
| Connecticut | 200 | 8 |
| Kentucky | 10 | 1 |
| Massachusetts | 14 | 24 |
| Minnesota | 50[a]<br>30[b] | 0.5<br>0.5 |
| Missouri | 500[a]<br>30[b] | 0.5<br>0.5 |
| Montana | 50[c] | 1 |
| Nevada | 240 | 8 |
| New York | 10 | 1 |
| North Dakota | 200[d]<br>100[c] | 1<br>24 |
| Oklahoma | 100 | 0.5 |
| Pennsylvania | 100 | 1 |
| Rhode Island | 10 | 1 |
| Texas | 80 | 0.5 |
| Virginia | 160 | 24 |
| Hawaii | 40 | 1 |
| Delaware | 30 | 1 |
| Indiana | 50 | 1 |

[a]Not to be exceeded more than two times/year.
[b]Not to be exceeded more than two times/five consecutive days.
[c]Not to be exceeded more than one time/year.
[d]Not to be exceeded more than one time/month.

and California were reviewed in greatest detail because they are major oil and gas producing States. These states have extensive regulations dealing with $H_2S$ in the oil and gas industry. California's air quality program is managed by 33 independent air pollution control districts and its Division of Oil and Gas is divided into 6 districts where District heads have great flexibility in enforcing rules. Therefore, California's program is discussed in the greatest detail.

**Selected Oil and Gas Producing States**

Oklahoma

The $H_2S$ regulations for Oklahoma (10.3.16, "Operation of Hydrogen Sulfide Areas") were listed in *Guidelines for Petroleum Emergency Field Situations in the State of Oklahoma*, a guidance manual that expands on the regulations. The guidance manual contains sections on characteristics and effects of $H_2S$, recommended guidelines for safe drilling and production operations in an $H_2S$ environment.

The following agencies regulate oil and gas activities in Oklahoma:

- Oklahoma Corporation Commission (OCC), Oil and Gas Conservation Division
- Oklahoma Air Quality Service
- Osage Indian Tribe (OIT)
- U.S. Bureau of Land Management
- U.S. Environmental Protection Agency.

The OCC has jurisdiction over laws and regulations "relating to the conservation of oil and gas and the prevention of pollution in connection with the exploration, drilling, producing, transporting, purchasing, processing and storage of oil and gas..." (OCC, 1986). The OIT has sole jurisdiction regarding oil and gas operations in Osage County. The U.S. Bureau of Land Management has responsibility for cases where both surface and mineral rights are owned by the Bureau or by an Indian tribe other than the Osage Tribe.

As noted earlier in this chapter, Oklahoma has an $H_2S$ ambient air quality standard. This regulatory program (administered by the Air Quality Service) is used to control routine emissions (through permit) from oil and gas facilities.

The accidental release of $H_2S$ from facilities is regulated by the OCC. Rule 165:10-3-16 of the OCC rules requires operators to assess their facilities for $H_2S$ release potentials that would cause harm to the public. The rule is applicable to all facilities that handle natural gas containing $1 \times 10^5$ ppb $H_2S$ or more and have a significant radius of exposure to cause adverse effects on the public with the exception of storage tanks. The "radius of exposure" is that distance from a source where the ground level concentration of hydrogen sulfide resulting from a release of gas from a facility is $1 \times 10^5$ ppb or $5 \times 10^5$ ppb whichever is applicable in the Rule. The Rule applies as follows:

- Does the facility (drilling, producing, injection, storage, etc.) handle hydrocarbon fluids containing $1 \times 10^5$ ppb $H_2S$ or more? If yes;

- Determine the $1 \times 10^5$ ppb radius of exposure using an equation required in the Rule or other methods approved by the Commission. The $H_2S$ escape rate from the facility must be determined as required by the Rule.

- If the $1 \times 10^5$ ppb radius of exposure is in excess of 50 feet, warning, marker and security provisions must be provided at the facility.

- If the $1 \times 10^5$ ppb radius of exposure is in excess of 50 feet and includes a public area or if the $5 \times 10^5$ ppb radius of exposure is in excess of 50 feet and includes a public road or if the $1 \times 10^5$ ppb radius of exposure is in excess of 3000 feet, control and safety equipment and a contingency plan must be provided for the facility.

- Facility storage tanks near atmospheric pressure containing $5 \times 10^5$ ppb or greater $H_2S$ must have warning signs, wind indicators and possible fencing. Radius of exposure calculations are not applicable to storage tanks.

- $H_2S$ training, injection or flaring provisions, accident notification and other requirements are addressed in the Rule (personal communication, W. Freeman, Shell Oil, 6/23/93).

The OCC does not keep an emissions inventory of accidental $H_2S$ releases, but it does keep an inventory of wells with actual or potential $H_2S$ problems. Furthermore, an inventory of inspection data is kept by individual inspectors in the State and the local field offices. Any emissions of $H_2S$ exceeding the OCC standard of $2.5 \times 10^4$ ppb must be reported to the OCC by the emitting facility. Rule 3-2032, $H_2S$ Operation, is intended to provide for the protection of the public's safety in areas where $H_2S$ concentrations greater than $1 \times 10^5$ ppb may be encountered.

Drilling facilities are not required to submit data periodically to show that they are in compliance with regulations. Facilities report release of $H_2S$ on an "honor system" once permits are granted. When noncompliance is discovered, the OCC can use administrative proceedings to shut down or fine the operation. However, in recent years, there has been no evidence of noncompliance with the $H_2S$ regulations.

The OCC lists training requirements for employees who will work in areas of potential $H_2S$ exposure. The training must cover hazards and characteristics of $H_2S$, operation of safety and life support systems, and emergency response procedures. OCC safety inspectors attend annual industry-sponsored training programs in order to stay current on safety developments and to check the safety of their breathing equipment. Each $H_2S$ inspector is required to have an $H_2S$ monitor, a manual, $H_2S$ gas monitoring test tubes, and a

self-contained air breathing apparatus. Specific $H_2S$ provisions also exist regarding $H_2S$ detection and alarm equipment, accident notification, injection, and flaring. In 1991, the OCC and the industry jointly sponsored an $H_2S$ safety seminar. A film about $H_2S$ safety was presented to regulatory and industry personnel, and questions about $H_2S$ safety were answered. Safety training has also been provided to local police, fire, sheriff and ambulance services, and to interested oil and gas operators, as requested.

The enforcement, field monitoring, and inspection departments of the OCC employ 69 people. The State currently has two $H_2S$ inspectors and a third is anticipated. In 1991, one emergency involving the accidental release of $H_2S$ was reported to the OCC. However, the accident, which resulted in the death of one worker, was not related to the extraction of oil and gas resources.

Texas

Six agencies regulate oil and gas activities in Texas:

- Railroad Commission of Texas
- Texas Water Commission
- Texas Air Control Board
- Texas Parks and Wildlife Department
- U.S. Army Corps of Engineers
- U.S. Environmental Protection Agency.

The Railroad Commission regulates most of the operations of the oil and gas industry but has no authority over the Clean Air Act Amendments. The Railroad Commission is responsible for the well spacing, construction requirements (casing etc.), and most aspects of environmental protection and works with other State Agencies to ensure that their concerns are addressed. The Texas Water Commission works with the Railroad Commission on water quality issues. The Texas Air Control Board has jurisdiction over the regulation of oil field activities that generate air emissions. The Texas Parks and Wildlife Department investigates fish kills and water pollution complaints and evaluates the effects of discharged wastes on fish and wildlife. The Railroad Commission has jurisdiction over all oil and gas activities on Federal lands in Texas, regardless of who owns the mineral rights. The U.S. Army Corps of Engineers has permitting responsibility for activities that would affect statutory wetlands.

The Texas Air Control Board (TACB) is responsible for enforcing the Texas ambient air quality standard for $H_2S$ (discussed previously). Certain allowances are made from the air standard if the hydrogen sulfide affects only property used for other than residential, recreational, business, or commercial purposes, such as industrial property and vacant tracts and range lands not normally occupied by people (i.e., the emission limit is raised to 120 ppb/30 min). If an operator violates these ambient air levels, corrective action must be taken such as flaring, installation of vapor recovery, etc. Consequently, the unauthorized emission of $H_2S$ that exceeds the time weighted averages for the land use discussed above is a

violation of regulation and must be addressed by the operator. In addition, the TACB requires permits for facilities that handle sour gas emissions from crude oil storage which also address emergency releases from these type facilities.

Texas regulations on $H_2S$ for drilling, extraction, and abandonment are listed under Statewide Rule 36 - Hydrogen Sulfide Safety, Section 3.36 (051.02.02.036, "Oil, Gas, or Geothermal Resource Operation in Hydrogen Sulfide Areas," as amended September 1, 1976). The Hydrogen Sulfide Safety Rule in Texas—issued to address accidental releases—applies to facilities that could expose the public to concentrations of $H_2S$ in excess of $1 \times 10^5$ ppb as a result of an accidental release. Operators handling hydrocarbon fluids containing $1 \times 10^5$ ppb or more $H_2S$ must determine if the Rule applies to their facility. If it does, they must calculate the radius of exposure; determine if the public will be impacted; and, if so, install warning signs, ensure security measures, address storage tank requirements, install appropriate safety equipment, develop contingency plans, provide training and implement other requirements as necessary. In addition, all operators subject to Rule 36 must submit a Certificate of Compliance to the Railroad Commission to demonstrate that they have complied with these requirements. This rule requires that employees working in $H_2S$ areas be trained in the characteristics and effects of the gas. The Railroad Commission of Texas publishes a training manual containing this information. The Texas and Oklahoma regulations are virtually identical. Most of the Texas regulations were discussed in the previous section on Oklahoma regulations. The Hydrogen Sulfide Safety Rule in Texas does require safety equipment, alarm equipment, monitors, etc., but does not specify exact types in an attempt to remain flexible and allow for new technology. It was designed for the protection of the general public rather than industry, since OSHA rules are designed to protect industry workers (personal communication, W. Freeman, Shell Oil, 6/23/93).

In Texas, the Railroad Commission does keep an emissions inventory on accidental $H_2S$ releases. Any emissions of $H_2S$ that are found to be of sufficient volume to present a hazard and/or any $H_2S$-related accidents must be reported to the Railroad Commission by the emitting facility. Operator certificates are required by the Railroad Commission to demonstrate that prevention and response measures have been taken to address accidental releases of $H_2S$.

There was one case of noncompliance during 1991, which involved natural gas leaking from a pipeline. The Railroad Commission canceled the Certificate of Compliance for the operators of the well, which prevented the facility from producing or selling the product until the leak was fixed. In 1991, there were emergencies involving the accidental releases of $H_2S$. Those accidents were discussed in Chapter III.

The enforcement, field monitoring, and inspection departments of the Railroad Commission employ 215 people. Ground testing for traces of $H_2S$ is performed near the wells. Emission data on each well are submitted to the Railroad Commission using the Form of Compliance. When noncompliance is discovered, the Commission uses administrative

proceedings to implement the following enforcement actions: enforcement letter, pipeline severance, zero allowable emissions, sealing, permit revocation and/or administrative penalties. The Railroad Commission may also seek civil penalties through the Attorney General's Office.

Michigan

The Michigan regulatory program is published in *Michigan's Oil and Gas Regulations - Act 61* (P.A. 1939 as amended and promulgated rules - Circular No. 15, revised in 1987, MDNR). Most of the regulations in the Michigan guidance were covered in the sections on Texas and Oklahoma regulations.

A review of *Michigan's Oil and Gas Regulations* reveals that the State has a comprehensive set of regulations dealing with $H_2S$. The Michigan rules require extensive training for all employees and contractors involved in drilling, completing, testing, producing, repair, workover or service operations. Employees must receive training in the following areas: physical properties and physiological effects of $H_2S$, effects of $H_2S$ on metals and elastomers, emergency escape procedures, location and use of safety equipment, the location and operation of detection and warning systems and the location of primary and secondary briefing areas. Briefing areas are defined in *Michigan's Oil and Gas Regulations* as the areas "nearby where personnel can assemble in case of an emergency." Michigan defines safety equipment as including items such as first aid kits, dry chemical fire extinguisher, ropes, flare guns, portable $H_2S$ detectors and warning signs.

In addition to training requirements, the Michigan oil and gas regulations contain comprehensive rules for the preparation of a contingency drilling plan in order to provide a plan for alerting and protecting personnel and the public in case of an emergency.

Five agencies regulate oil and gas activities in Michigan:

- Michigan Department of Natural Resources (MDNR)
- Michigan Department of Commerce, Public Service Commission
- U.S. Forest Services
- U.S. Bureau of Land Management
- U.S. Environmental Protection Agency.

The Department of Natural Resources is responsible for the well spacing, construction requirements (casing, etc.), and most aspects of environmental protection. The Michigan Public Service Commission regulates the production of gas from dry natural gas reservoirs and the safety of gas pipeline construction. When dealing with split estate situations, the U.S. Forest Service will issue a Special Use Permit which allows an operator to drill within the forest boundary. When both the forest surface and corresponding mineral rights are Federally owned, the U.S. Bureau of Land Management (BLM) issues drilling permits and the U.S. Forest Service issues Surface Use Plans. The BLM issues drilling permits in all

cases related to onshore Federal mineral estates (personal communication, T. Alexander, DOE, 2/22/93).

Worker safety issues are the responsibility of the Michigan Department of Labor. Part 57 of the General Industry Safety Standards Commission Safety Standards deals with oil and gas drilling operations safety standards. Under Rule 5717(1), the drilling and servicing of wells containing $H_2S$ shall be conducted as prescribed in the American Petroleum Institute's Recommended Practice No. 49 (API, 1987).

The MDNR's Air Quality Division regulates $H_2S$ emissions from all sources in the oil and gas industry. Rule 336.1403 states: "It is unlawful for a person to cause or allow the emission of sour gas from an oil or natural gas producing or transporting facility or a natural gas processing facility without burning or equivalent control of hydrogen sulfide and mercaptans." The Rule does allow operators with stripper wells to emit small quantities of $H_2S$ unless one complaint is received from the public which would require some type of abatement technique to be imposed. All facilities handling $H_2S$ are subject to these regulations.

The Geological Survey Division (GSD) of the Department of Natural Resources regulates accidental releases of $H_2S$ in the oil and gas industry. In addition, it overlaps with the Air Quality Division on emission controls at production facilities. It appears that two agencies in the MDNR regulate $H_2S$ handling facilities. Under Rule 299.1911-1939, operators handling hydrocarbon fluids containing more than $3 \times 10^5$ ppb $H_2S$ must define a Well Class (defined by the radius of exposure in Rule 299.1912) to determine the applicability of the Rule. The radius of exposure is defined using the same dispersion equation as Texas Rule 36. The Rule addresses equipment standards, location standards for drilling and production equipment, contingency planning, training, drilling, testing, production operations, servicing operations and nuisance odor requirements (personal communication, W. Freeman, Shell Oil, 6/23/93).

The enforcement, field monitoring, and inspection departments for oil and gas regulation by the Geological Survey Division (GSD) of the MDNR employ 47 people. Wells are retested one year after the initial well test was performed, to check for compliance with laws. Further periodic tests are required only at the request of the MDNR. When a well is not in compliance, the MDNR can use administrative proceedings to shut down drilling processes and production, stop issuing permits to drill, stop well ownership transfers, and issue fines. Fines are also issued for falsifying records required by the GSD enabling legislation (Act 61, P.A. of 1939, amended). Violation of the Act or a rule or order under the Act carries a penalty of not more than $1,000.00 per day that the violation continues. In 1991, there was no evidence of noncompliance for the release of $H_2S$.

The MDNR does not keep an emissions inventory of the accidental releases of $H_2S$ from well blowouts and flare gas releases. Emissions of $H_2S$ are reported by industry personnel to MDNR field personnel, who may keep records on the releases. One incident

was reported to the MDNR in 1990, which involved a pumper who was working on a storage tank. The exact date and nature of the incident were not available.

California

The following agencies regulate oil and gas activity in California:

- California Department of Conservation, Division of Oil and Gas
- California Water Resources Control Board and the nine Regional Water Quality Control Boards
- California Department of Health Services
- California Department of Fish and Game, Office of Spill Prevention and Response
- California/EPA Department of Toxic Substances Control
- California State Fire Marshall's Office
- California Public Utilities Commission
- California OSHA
- California Air Resources Board and the county or multi-county regional Air Pollution Control Districts
- California Governor's Office of Emergency Services
- State Lands Commission
- California Coastal Commission
- Local government agencies
- U.S. Bureau of Land Management
- U.S. Department of Energy
- U.S. Environmental Protection Agency.

The Division of Oil and Gas of the California Department of Conservation is responsible for the management and conservation of oil and gas resources. The Division issues permits for and inspects the drilling, reworking, and abandonment of oil and gas wells. Under delegated authority from the EPA, the division also issues underground injection control well permits for Class II injection wells.

Division 3 - Oil and Gas, part of the California Code of Civil Procedure, contains the California laws for conservation of petroleum and gas (CDC, 1991). Table IV-2 highlights key sections of the law applicable to $H_2S$ releases. Although, there is no quantitative limit to $H_2S$ emissions, the law grants the supervisor of the Oil and Gas Division, discretionary authority to control $H_2S$ releases to ensure protection of human health and the environment.

California's Code of Regulations contains the oil and gas regulatory program enforced by the Division of Oil and Gas. These regulations are highlighted in Table IV-3. These rules include the definition of the term "critical well," requirements for contingency plans,

**Table IV-2. Highlights of California Laws for Conservation of Petroleum and Gas Pertaining to $H_2S$ Emissions**

| Ch., Art., Section | Subject | Description |
|---|---|---|
| 1, 4, 3219 | Blowout prevention | Where high-pressure gas exists, use adequate casing and safety devices |
| 1, 4, 3224 | Order for repair | Authorizes supervisor to order tests or repairs needed to prevent damage to life, health, natural resources, etc. |
| 1, 4, 3228 | Abandonment of wells | Protects ground and surface water from gas-bearing strata |
| 1, 4, 3235 | Complaint | Authority to investigate complaints |
| 1, 4, 3236 | Penalty | For obstructing enforcement, $100 - $1,000 or up to 6 months imprisonment per offense |
| 1, 4.1, 3241 | Strategy to extract gas in high risk areas | Develop strategy to extract hazardous gases from abandoned wells to protect public health and safety |
| 1, 4.2, 3251 | Define "hazardous well" | Poses danger to life, health, or natural resources |
| 3, , 3600 | Spacing wells | Well must be at least 100 feet from parcel's boundary or public road |

rules include the definition of the term "critical well," requirements for contingency plans, and environmental protection.

The Division of Oil and Gas has also published a guidance document on $H_2S$, *Drilling and Operating Oil, Gas, and Geothermal Wells in an $H_2S$ Environment* (Dosch and Hodgson, 1986). This guidance document reflects the American Petroleum Institute's publication RP 49, *Safe Drilling of Wells Containing Hydrogen Sulfide* (API, 1987) and recommends safety procedures for $H_2S$ release scenarios. The California Division of Oil and Gas (CDOG) is divided into six districts. Figure IV-1 shows the six districts and the distribution of $H_2S$ in California, presenting parts per million of $H_2S$ gas in some California oil and geothermal fields. Table IV-4 shows the documented concentration by oil field in each district. Three of the districts are discussed here.

District 1 of the Division of Oil and Gas has three oil and gas inspectors and seven energy engineers who inspect well drilling and rework operations. The inspectors wear tri-gas monitors ($H_2S$, oxygen, and combustibles). The well-permitting program does not specify $H_2S$ limits. All wells are inspected at least once a year. Idle wells must be pressure-tested periodically to minimize casing leaks. Steam flooding, an enhancement process that often creates $H_2S$, is used frequently in the district. District 1 authorities know of past $H_2S$ incidents leading to human injuries; however, because records are not computerized, exact data are not available (personal communication, K. Carlson, CDOG, 8/27/92).

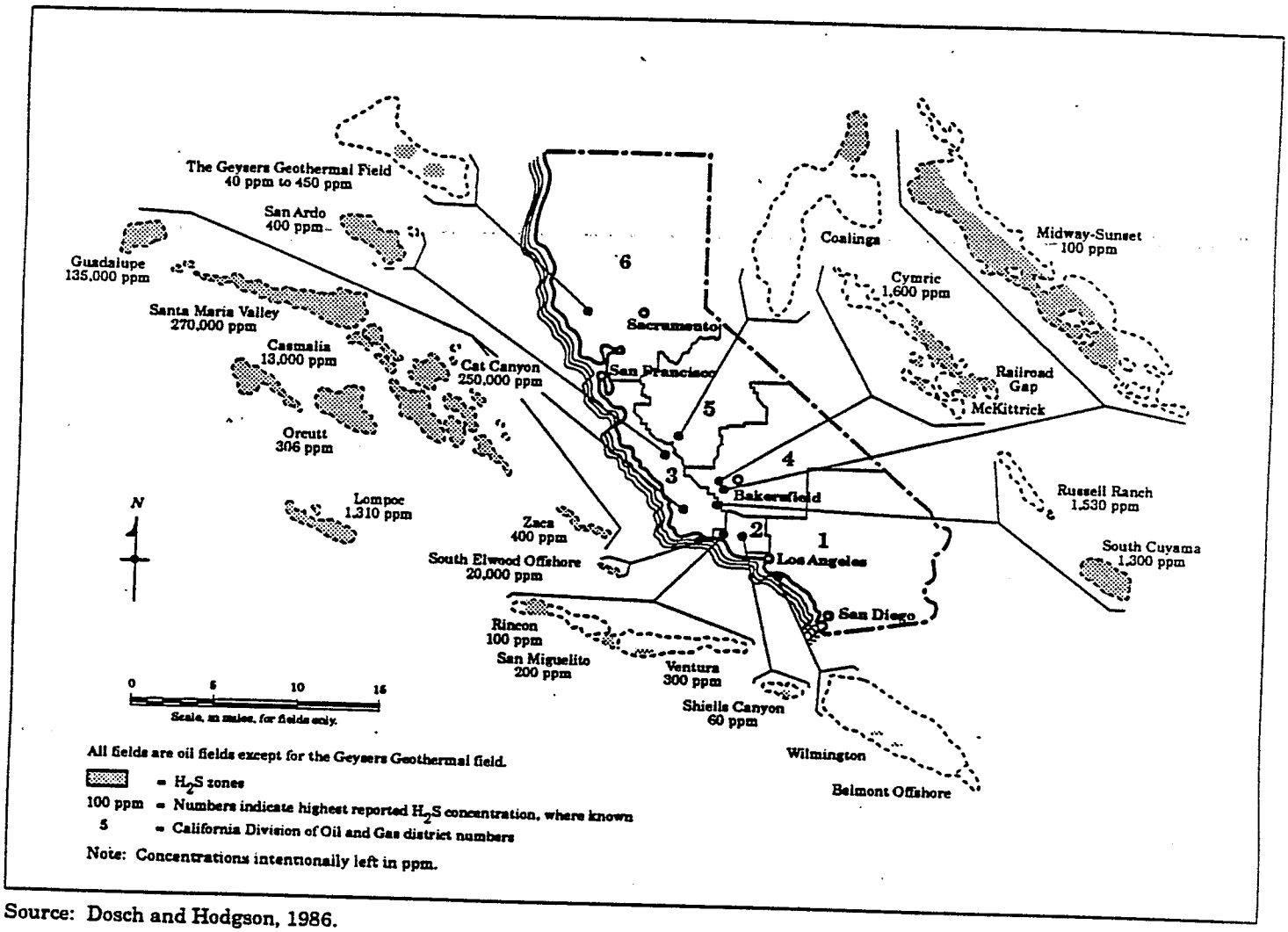
District 3 has 1,929 producing wells and 2,845 shut-in wells (i.e., no production is made on the well; its pump is turned off, the stuffing box is closed, and it is inspected to ensure no leakage). Three field inspectors cover District 3 (personal communication, A. Kollar, CDOG, 8/28/92).

District 4, which includes Kern County, has nine field inspectors, each equipped with an "escape pack" for $H_2S$ protection. An environmental inspection is performed for every lease on every well. The inspection covers the surface area, well condition, tank condition, and operation. There are more than 40,000 wells in Kern County alone. District 4 had no records of $H_2S$ incidents. However, inspectors in Kern County/San Joaquin Air District (described below) have documented incidents of $H_2S$ releases (personal communication, R. Bowles, CDOG, 8/27/92).

The California Air Resources Board is authorized to enforce a statewide ambient air quality limit for $H_2S$ emissions of 30 ppb over one hour's averaging time. However, California's air quality program is managed on a smaller scale by the 33 county or multi-county air pollution control districts (APCDs) shown in Figure IV-2 (CA Air Resources Board, 1991). Each district acts as an independent regulatory agency, establishing and

**Table IV-3. Highlights of Title 14, Chapter 4 of the California Code of Regulations – Development, Regulation, and Conservation of Oil and Gas Resources**

| Article, Section | Subject | Description |
|---|---|---|
| Subchapter 1 | | |
| 1, 1712 | Scope | Onshore drilling and production; grants Oil and Gas Division Supervisor authority to establish field rules |
| 2, 1720 | Critical well | Addresses distances to public areas and navigable waters |
| 2.1, 1721 | Well spacing | Objectives include protecting public health, safety, welfare and the environment |
| 3, 1722 | General | Good oilfield practices, blowout prevention and control plan, prompt reporting of significant gas leaks |
| 3, 1724.3 | Well Safety Devices | Required of certain critical wells |
| 3, 1724.4 | Testing/inspecting Safety Devices | Test at least every 6 months |
| Subchapter 2 | Environmental Protection | Requires covers on well cellars, no excessive leakage including wellheads and pipelines |




Source: Dosch and Hodgson, 1986.

**Figure IV-1. Parts per million of $H_2S$ gas in some California oil and geothermal fields. Data compiled in 1976.**

Table IV-4. $H_2S$ in California Oil, Gas, and Geothermal Fields

| Oil and Gas District | Fields with $H_2S$ Concentrations $1x10^5$ ppb or Above | Fields with $H_2S$ Concentrations Under 100 ppm | Fields with $H_2S$ Odor, But With Concentrations Unknown |
|---|---|---|---|
| 1 | — | — | Wilmington, Huntington Beach, Newport, Torrance, Brea Olinda |
| 2 | Rincon, $1 \times 10^5$ ppb<br>San Miguelito, $2 \times 10^5$ ppb<br>Ventura, $3 \times 10^5$ ppb | Shiells Canyon 60 ppm | Aliso Canyon, Bardsdale, Big Mountain, Del Valle, Las Llajas, Oak Park, Oakridge, Ojai, Piru, Santa Paula, Santa Susana, Simi, South Mountain, Tapo Canyon So., Temescal, Torrey Canyon, and West Mountain |
| 3 | Casmalia, $1.3 \times 10^7$ ppb<br>Cat Canyon, $2.5 \times 10^8$ ppb<br>Cuyama So., $1.3 \times 10^6$ ppb<br>Elwood So., Offshore, $2 \times 10^7$ ppb<br>Guadalupe, $1.35 \times 10^8$ ppb<br>Lompoc, $1.31 \times 10^6$ ppb<br>Orcutt, $3.06 \times 10^5$ ppb<br>Russell Ranch, $1.53 \times 10^6$ ppb<br>San Ardo, $4 \times 10^5$ ppb<br>Santa Maria Valley, $2.7 \times 10^8$ ppb<br>Zaca, $4 \times 10^5$ ppb | — | Capitan Onshore, King City<br>Four Deer |
| 4 | Midway Sunset, $1 \times 10^5$ ppb<br>Cymric, $1.6 \times 10^6$ ppb | — | North Belridge, South Belridge, Blackwells Corner, Edison, Northeast Edison, Kern River, Lost Hills, McKittrick, Mount Poso, Poso Creek, Railroad Gap, and Wheeler Ridge |
| 5 | — | — | Coalinga |
| 6 | — | — | — |
| Geothermal District | | | |
| G3 | The Geysers, $4x10^4$ - $4.5x10^6$ ppb | — | — |

$H_2S$ in some California oil and geothermal fields. Data compiled in September 1976. (Data in the first two columns are on Figure IV-1).

Source: Dosch and Hodgson, 1986.

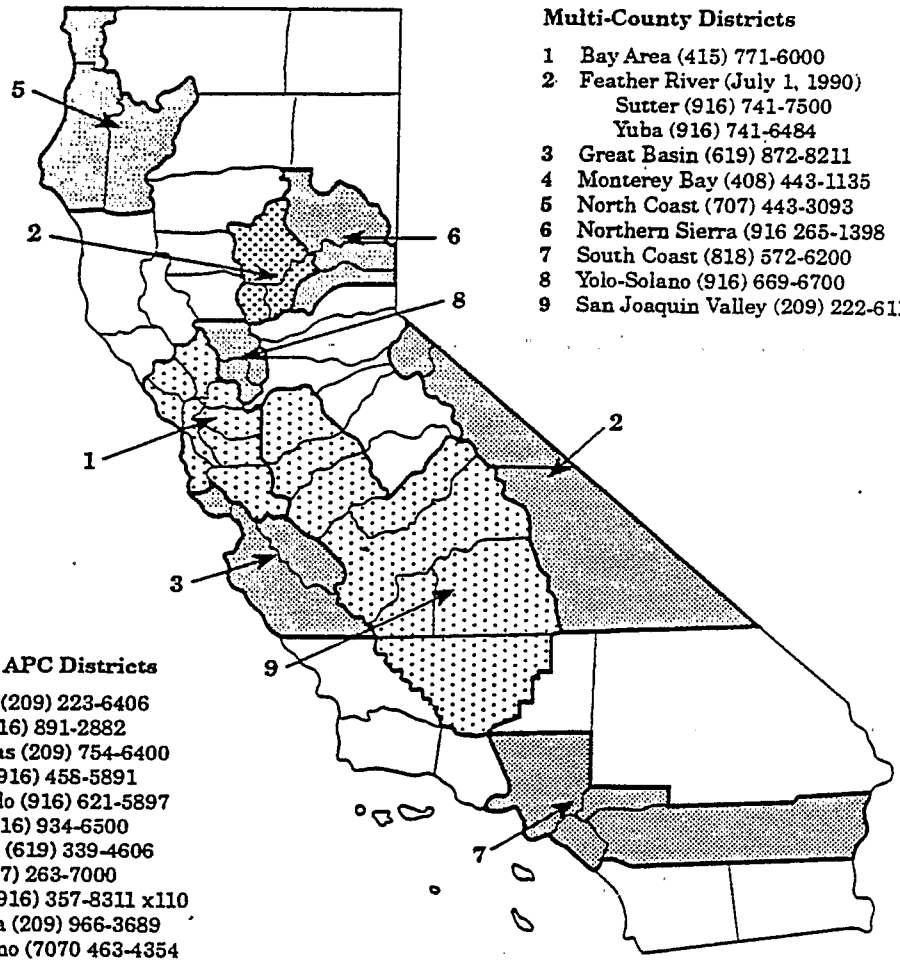
enforcing air quality rules tailored to the district's needs. Districts with significant oil production are:

Ventura County
Santa Barbara County
San Joaquin Unified Air District
South Coast Air Quality Management District
Monterey Bay Unified Air District
Bay Area Air Quality Management District.

This report highlights $H_2S$ emissions programs in four districts: Ventura County, Santa Barbara County, San Joaquin Unified Air District, and the South Coast District.

Ventura County has Rule 54, "Sulfur Compounds," in place for air emissions containing sulfur compounds. This rule, adopted in 1968 and most recently revised in 1983, includes a limit for $H_2S$ not to exceed $1 \times 10^4$ ppb by volume at the point of discharge. The point of discharge includes any distinguishable emission point such as valves, flanges, or process vents. There are no control technology regulations for $H_2S$ in Ventura County other than these equipment standards. Another $H_2S$ rule requires that the aboveground average concentration at or beyond the property boundary shall not be in excess of 60 ppb for over 3 minutes. The Ventura County limits were adopted in 1968 when the APCD was formed. Natural emissions of $H_2S$ are low in the county's oil well fields, and $H_2S$ monitoring is only performed when a problem is suspected (i.e., when the odor is detected). The APCD uses hand-held monitoring devices to inspect problem areas. No routine monitoring records are kept on file in Ventura County, but wells are inspected at least once a year, with large wells inspected more frequently (personal communication, K. Duval, Ventura APCD, 8/29/92). Ventura County has an enforcement staff of about 20 people, including 8 field inspectors (personal communication, K. Duval, Ventura APCD, 11/23/92).

Emission standards in Santa Barbara County are basically the same as in Ventura County. However, tighter emission limits are applied in parts of the county with $SO_2$ (an oxidation product of $H_2S$) nonattainment areas. Rule 309, "Specific Contaminants," for Santa Barbara County states that sulfur recovery units shall not emit more than $5 \times 10^5$ ppb as $SO_2$ or $1 \times 10^4$ ppb as $H_2S$. Rule 310 for odorous organic sulfides states that concentrations of organic sulfides beyond the property boundary shall not exceed 60 ppb/3 minutes or 30 ppb/hr. For gas produced and used as fuel in equipment on a well site, the sulfur content limit in the county's northern air shed is $7.96 \times 10^5$ ppb sulfur; in the southern county air shed, the limit is $2.5 \times 10^5$ ppb. Control technologies are not used on well heads for $H_2S$ emissions. However, controls do exist for volatile organic compound (VOC) emissions from well fittings, stuffing boxes, well cellars, sumps and pits. Rules are being developed to require these controls, primarily in the surface area of the well cellar to control the release of VOC. This technology will also control $H_2S$ emissions indirectly. The county's 10 field inspectors inspect wells for all types of emission sources at least once a year. $H_2S$ violations via the total sulfur emission limit are not a problem because by the time




**Multi-County Districts**

1 Bay Area (415) 771-6000
2 Feather River (July 1, 1990)
Sutter (916) 741-7500
Yuba (916) 741-6484
3 Great Basin (619) 872-8211
4 Monterey Bay (408) 443-1135
5 North Coast (707) 443-3093
6 Northern Sierra (916 265-1398
7 South Coast (818) 572-6200
8 Yolo-Solano (916) 669-6700
9 San Joaquin Valley (209) 222-6111

**County APC Districts**

Amador (209) 223-6406
Butte (916) 891-2882
Calaveras (209) 754-6400
Colusa (916) 458-5891
El Dorado (916) 621-5897
Glenn (916) 934-6500
Imperial (619) 339-4606
Lake (707) 263-7000
Lassen (916) 357-8311 x110
Mariposa (209) 966-3689
Mendocino (7070 463-4354
Modoc (916) 233-3939 x401
No. Sonoma (707) 433-5911
Placer (916) 889-7130
Sacramento (916) 386-6650
San Bernardino (619) 243-8920
San Diego (619) 694-3307
San Luis Obispo (805) 549-5912
Santa Barbara (805) 961-8800
Shasta (916) 225-5674
Siskiyou (916) 842-8029
Tehama (916) 527-4504
Tuolumne (209) 533-5693
Ventura (805) 654-2806

Source: California Air Resources Board, 1991.

**Figure IV-2. Multi-county districts.**

the ambient air quality standard is exceeded, the operator has already been alerted to a safety problem and is responding. The county has seven currently active $H_2S$ ambient monitoring stations; however, these are at oil and gas processing facilities, rather than at well fields (personal communication, J. Top, St. Barbara APCD, 8/20/92).

The San Joaquin Unified Air District enforces Rule 407, "Sulfur Compounds," which limits the emission concentration of sulfur compounds at the point of discharge to 0.2 percent volume calculated as $SO_2$ (or $2 \times 10^6$ ppb $SO_2$). This rule, adopted in 1972 and renumbered in 1989, applies to any gas line or vapor control line from a well. Rule 220.1, "New and Modified Stationary Source Review Rules," has a trigger value for $H_2S$ or total reduced sulfur or sulfur compounds other than $SO_x$ of 54.79 lb/day. If this value is exceeded, the responsible party must use Best Available Control Technology (BACT) on the emission source. Rule 220.1 was adopted in September 1991 and revised March of 1992.

The San Joaquin District does not look at or enforce $H_2S$ regulations until the $2 \times 10^6$ ppb $SO_2$ emission limit is exceeded, because the rule is based on the impact of $SO_2$ on human health and the environment, not on the health effects of $H_2S$. No ambient monitoring of $H_2S$ is required by the district. However, the oil companies are required to keep their own records of $SO_2$ monitoring for two years. Companies also have $H_2S$ monitoring data, and the State has the authority to request these data at any time (personal communication, M. Amundsen, San Joaquin, 8/21/92).

Kern County, part of the San Joaquin Unified Air District, has three of the largest producing wells in the United States. The county's production volume is exceeded only by Alaska, Texas, and Louisiana. The wells in Kern County produce a unique heavy crude and some use steam injection to enhance pumping. $H_2S$ is a problem in well fields in the county, where numerous stripper wells (defined in Chapter II) are operating. The county has a ten-person enforcement team that performs inspections at least once a year. Steam casing collection systems, valves, fittings, etc., are inspected by staff wearing $H_2S$ monitors. Inspectors in Kern County have been exposed to $H_2S$ in the field. In one case, an inspector was exposed to greater than $1 \times 10^6$ ppb. The case involved a report from a fire department station downwind of a well and complaints of odor and illness. $H_2S$ was measured at the station at $5 \times 10^4$ ppb. The source was a leaking underground gas recovery line. Companies are required to keep records of such incidents and report them to CAL OSHA (personal communication, M. Amundsen, San Joaquin Unified Air District, 8/21/92).

During conversations with Kern County representatives, it was noted that an important control technology for $H_2S$ at wells is a casing collection system, which can be added to collect natural gas containing $H_2S$ that has built up in the casing over time. If the natural gas pressure is not relieved, well production is hindered. Companies tend to release this gas to the atmosphere, but a casing collection system can treat the gas by vapor incineration (98 to 99 percent hydrocarbon destruction efficiency). However, the economic incentive to put casing collection systems on stripper wells is normally low due to the low

volume of oil produced (personal communication, M. Amundsen, A. Phillips, San Joaquin, 8/21/92).

The South Coast Air Quality Management District has no specific regulations pertaining to $H_2S$ or oil production. Rules in place that indirectly control $H_2S$ emissions include Rule 431.1, "Sulfur Content of Gaseous Fuels," which states that, effective May 1994, natural gas cannot be burned or sold for burning if it contains greater than $4 \times 10^4$ ppb total sulfur. This rule also requires organic vapor recovery systems, which would recover any $H_2S$ gas along with the volatile organics. Rule 402 could also apply to $H_2S$, particularly for stripper wells that are too small for permitting. This rule is a nuisance rule that could be used to close wells if, for example, neighbors complained about $H_2S$ odors or other health effects (personal communication, C. Bhatti, South Coast AQMD, 8/25/92). The South Coast District's enforcement program is managed as part of the Stationary Source Compliance Office, which has a staff of 500 (personal communication, C. Bhatti, South Coast AQMD, 11/23/92).

California's Occupational Safety and Health Administration is authorized to administer the Federal OSHA program. There are two OSHA standards that apply to $H_2S$. One focuses on the maintenance and use of valves. The second is the Permissible Exposure Limit for $H_2S$. It is difficult to monitor compliance with this limit because operations are outdoors. CAL OSHA maintains a database of occupational accidents. No accidents were found in the database related to $H_2S$ releases at California oil wells dating back to 1982 (personal communication, R. Hayes, CAOSHA, 9/11/92). However, $H_2S$ incidents were recorded in some of the Air Pollution Control Districts and Division of Oil and Gas Districts (personal communication, M. Amundsen, San Joaquin Unified Air District, 8/21/92).

The California Water Resources Control Board is generally responsible for the protection of the State's waters and for preserving all present and anticipated beneficial uses of these waters. The California Department of Health Services is responsible for the regulation of hazardous wastes. It determines which waste streams and constituents are hazardous under California's laws. The State Land Commission has joint responsibility with the Division of Oil and Gas for wells on State-owned, onshore lands.

The Office of Emergency Services administers Chapter 6.95 of the California Health and Safety Code which states that every business handling any hazardous material greater than 55 gal., 500 lb. or 200 cubic feet (gaseous material) must register and develop an emergency response plan and business plan. If the business handles extremely hazardous substances onsite exceeding threshold planning quantities (500 lb for $H_2S$), a preliminary analysis of the facility must be made to determine if a significant risk potential exists for accidental release of the extremely hazardous substance. If the potential does exist, the facility must develop and submit a "risk management and prevention program" that addresses how to reduce or eliminate the potential for accidental release (personal communication, Dr. F. Lercari, Office of Emergency Services, 9/13/93).

A Comparison of $H_2S$ Regulatory Programs in Four States

Table IV-5 presents a summary of regulatory programs for $H_2S$ across California, Michigan, Oklahoma, and Texas. This summary addresses the area of "state ... control standards, techniques, and enforcement" designated for evaluation in Section 112(n)(5) of the Clean Air Act Amendments. Appendix B tabulates components of the States' regulatory programs in greater detail.

Texas, Oklahoma, and California have $H_2S$ ambient air quality standards in place. The California standard (30 ppb over 1-hr averaging time) is more stringent than the Texas standard (80 ppb over 0.5-hr averaging time) and the Oklahoma standard (100 ppb over 0.5-hr averaging time). Michigan does not have ambient air quality standards for $H_2S$.

The number of agencies in each State regulating oil and gas operations ranges from two in Oklahoma and Michigan to eleven in California. The enforcement staff, which includes inspectors and field monitoring staff, numbers 69 in Oklahoma, 215 in Texas, and 47 in Michigan. California's air emissions program is regulated by districts. The Santa Barbara District, an area with high concentrations of $H_2S$ in its oil fields, has 10 field inspectors who are also responsible for inspecting other commercial operations. Kern County, California, has a staff of 10 field inspectors who also have other inspection responsibilities.

Michigan, Oklahoma, and Texas each have $H_2S$-specific regulations related to public safety. In California, State law grants the Director of the Division of Oil and Gas discretion to require additional controls (for areas such as $H_2S$ emissions) on a case-by-case basis. However, none of the four States has specific $H_2S$ standards in place to protect the environment, i.e., ecological protection.

Of the four States reviewed, only Texas maintains an inventory of accidental releases of $H_2S$ from drilling and production operations. However, all four states require notification when threatening accidental releases occur. None of the four States requires reporting of $H_2S$ routine emissions. "Routine" excludes such incidents as vapor recovery unit failures and other equipment upsets.

Texas, Oklahoma, and Michigan require worker safety training for $H_2S$. California's Division of Oil and Gas, however, provides guidance on worker safety in the form of a publication (Dosch and Hodgson, 1986).

**Other Large Producing States**

The EPA gathered initial information on several State regulations and later contacted selected State agencies to obtain additional information on the unique aspects of the State regulations governing $H_2S$ emissions in the oil and gas industry. The results of each State review are summarized in the following sections.

**Table IV-5. A Comparison of Four States' $H_2S$ Regulatory Programs**

| $H_2S$ Area | Oklahoma | Texas | Michigan | California |
|---|---|---|---|---|
| Ambient air quality standard? | 0.10 (0.5 hr) | 0.08 (0.5 hr) | No | 0.03 (1 hr) |
| Number of State agencies regulating oil/gas | 3 | 4 | 2 | 6 |
| Size of enforcement/ inspection staff | 69 | 215 | 47 | * |
| Specific $H_2S$ regulations for: | | | | |
| Public Safety | Yes | Yes | Yes | No |
| Ecological Protection (administered by environmental agency) | No | No | Not clear | No |
| Inventory of accidental releases kept by State? | No | Yes | No | No |
| Routine reporting of emissions required? | No | No | No | No |
| Notification of a threatening accidental release? | Yes | Yes | Yes | Yes |
| $H_2S$ training required? | Yes | Yes | Yes | Guidance |

*Enforcement staff in California (example counties)

| | |
|---|---|
| Santa Barbara County Air Pollution Control District: | 10 |
| Kern County (in San Joaquin Unified Air District): | 10 |
| California Division of Oil and Gas – District 7: | 10 |
| California Division of Oil and Gas – District 4: | 9 |

Louisiana

Five agencies regulate oil and gas activity in Louisiana:

- Louisiana Department of Natural Resources, Office of Conservation
- Louisiana Department of Environmental Quality
- U.S. Bureau of Land Management
- U.S. Army Corps of Engineers
- U.S. Environmental Protection Agency.

The Louisiana Department of Natural Resources, Office of Conservation, regulates all subsurface and surface disposal of oil- and gas-associated wastes (*Statewide Order Governing the Drilling for the Producing of Oil and Gas in the State of Louisiana*). The office has primary responsibility for all classes of underground injection control wells. The Office of Conservation coordinates with the Louisiana Department of Environmental Quality, Office of Water Resources, on any problem dealing with discharges in the oil and gas industry. The U.S. Bureau of Land Management has jurisdiction over lease arrangements and post-lease activity on Federal lands where the mineral rights are Federally held. The Office of Conservation does not keep an emissions inventory for accidental $H_2S$ releases. Any emissions of $H_2S$ that exceed the Office of Conservation standard must be reported to the Office by the emitting facility.

The enforcement, field monitoring, and inspection departments of the Office of Conservation employ 34 inspectors. Emission data are sent to the Office of Conservation when an accidental release has occurred at the well site. The Office of Conservation, through administrative proceedings, can respond with the following enforcement actions when compliance is not met: compliance letters, compliance orders, civil penalty assessments, suspension/revocation of permits and pipeline severance.

In recent years, there has been no evidence of noncompliance and no emergencies involving the release of $H_2S$ from oil or gas wells. The drilling process is not a significant threat because underground sources of $H_2S$ are much deeper than the wells being drilled.

New Mexico

Five agencies have responsibilities for regulating oil and gas activities in New Mexico:

- New Mexico Oil Conservation Division of the Energy, Minerals and Natural Resources Department (OCD)
- New Mexico Oil Conservation Commission
- New Mexico Water Quality Control Commission
- U.S. Environmental Protection Agency

- U.S. Bureau of Land Management.

The Oil Conservation Division of the Energy, Minerals and Natural Resources Department is responsible for regulating oil and gas industry exploration and drilling, production, and refining. Its duties include regulating "nonhazardous" liquid and solid wastes from these operations to protect water quality, public health, and the environment. The Oil Conservation Commission works in conjunction with the Oil Conservation Division. The Commission initiates rules and orders to be administered by the Division. The Water Quality Control Commission develops water quality control standards and water pollution regulations. The U.S. Bureau of Land Management has jurisdiction over all Federally owned land, with the exception of Indian lands.

The Oil Conservation Division of Energy Resources (OCD) keeps emissions inventories at the district level. There are four districts in the State of New Mexico; any accidental release of $H_2S$ must be reported to the district division of the OCD. The enforcement, field monitoring and inspection departments of the OCD employ 18 people. Inspections are made by each district OCD office. In recent years, there has been no evidence of noncompliance with the $H_2S$ regulations set forth by the OCD, and no emergencies involving $H_2S$ have been reported.

New Mexico's Oil Conservation Commission Rule 118 is intended to provide for the protection of the public safety in areas where $H_2S$ concentrations greater than $1 \times 10^5$ ppb may be encountered. This rule adopts the guidance of the American Petroleum Institute publications RP 49 and RP 55 (discussed later in this chapter) and covers drilling, extraction, and abandonment.

North Dakota

Five agencies regulate oil and gas activities in North Dakota:

- North Dakota Industrial Commission, Oil and Gas Division
- North Dakota State Department of Health and Consolidated Laboratories
- U.S. Department of Agriculture, Forest Service
- U.S. Bureau of Land Management
- U.S. Environmental Protection Agency.

The North Dakota Industrial Commission, Oil and Gas Division, has regulatory authority over the drilling and production of oil, and is responsible for protecting the correlative rights of the mineral owners, preventing waste, and protecting all sources of drinking water. The Bureau of Land Management has jurisdiction over drilling and production on Federal lands, but the operator must obtain a permit from the Division of Oil and Gas. Drilling on forest land must comply with the rules of the U.S. Forest Service.

Any well completed or recompleted on or after July 1, 1987 must be registered with the State Department of Health and Consolidated Laboratories (NDSDH&CL). The registration process includes completion and submittal of a form which provides information about the well operator, well equipment (such as size and number of storage tanks, existence of a heater treater and type of fuel on which it is fired, flare stack height, etc.), surface equipment location, and disposition of produced gas. This form, submitted along with an analysis showing the $H_2S$ concentration of any produced gas, constitutes registration. Information derived from the registration is entered into a shared database, which is used by the North Dakota Industrial Commission's Oil and Gas Division, for storing production data; thus, an emissions inventory which represents actual emissions can be generated from the database for all registered wells. $H_2S$ concentrations in wellhead gas are field-pool specific; for example, within the Little Knife Oil Field, gas produced from the Madison Pool will have an $H_2S$ concentration of approximately 9.56 percent, gas produced from the Red River Pool will be approximately 7.91 percent $H_2S$, and gas produced from the Duperow and Bakken pools is likely to contain only negligible amounts of $H_2S$. $H_2S$ data from the registrations are, therefore, entered into the database as field-pool specific data (personal communication, D. Harman, NDSDH&CL, 5/19/93).

The enforcement, field monitoring, and inspection departments of the Division of Oil and Gas employ 14 people. The NDSDH&CL handles most of these complaint-related inspections. The Division of Oil and Gas can shut down an operation and fine up to $12,500 per day when compliance is not met. The NDSDH&CL can impose a fine and/or imprisonment.

$H_2S$ typically constitutes between 4 and 10 percent of the oil and gas found in North Dakota. Because of this prevalence, the State has established an ambient air quality standard (shown in Table IV-1).

The NDSDH&CL typically becomes more involved in situations where routine emissions (as opposed to catastrophic/episodical releases) from a production facility result in excessive ambient concentrations. This scenario typically manifests itself in the form of citizen complaints. In these situations, it has been the Department's experience that an equipment problem, such as flare stack ignitor malfunction (i.e., low efficiency flare), storage tank gasket degradation and leakage, etc., has been the primary cause. Correction of the immediate problem and implementation of a more rigorous maintenance schedule will typically resolve these cases (personal communication, D. Harman, NDSDH&CL, 5/19/93). Acute, unpredictable releases of $H_2S$, such as natural gas pipeline rupture, etc., are typically handled by the North Dakota Industrial Commission; however, the Industrial Commission has had no reports of emergencies involving accidental releases of $H_2S$ in the past two years.

Pennsylvania

Six agencies regulate oil and gas activities in Pennsylvania:

- Department of Environmental Resources,
- Bureau of Oil and Gas Management (BOGM)
- U.S. Environmental Protection Agency, Region III
- Pennsylvania Fish Commission
- U.S. Forest Service
- U.S. Bureau of Land Management.

The Bureau of Oil and Gas Management (BOGM) was created to coordinate and combine all regulatory activities of the oil and gas industry (*Oil and Gas Operators' Manual*). The U.S. Environmental Protection Agency issues permits for underground injection and secondary recovery. The Pennsylvania Fish Commission identifies pollution of surface waters and takes appropriate action under the Pennsylvania Fish and Boat Code.

The BOGM does keep records of any accidental releases; however, routine emission rates are not reported. Nearly all of Pennsylvania's $H_2S$ problems have occurred in the northern part of the State, around Lake Erie.

The enforcement, field monitoring, and inspection departments of the BOGM employ 38 people. The Department of Environmental Resources has the following enforcement options available when compliance is not met: notice of violation, citation for summary offense, misdemeanor, civil penalty, injunction, administrative order, consent order and agreement, permit suspension and/or revocation, and bond forfeiture.

Six wells near Lake Erie have significant concentrations of $H_2S$ that could be a threat to the surrounding environment and people. One incident in 1990 involved discharges of $H_2S$ from a well blowout. Local authorities evacuated a neighboring town until the $H_2S$ could be contained and the well plugged. The blowout did not cause any negative health effects or other types of injury.

In the past, Pennsylvania explored the possibility of establishing a committee that would include consultants, gubernatorial appointees, and citizens to examine $H_2S$ in relation to the oil and gas industry and determine if a serious problem exists. It is understood that this project is currently inactive due to budget limitations.

Wyoming

There are four agencies that regulate oil and gas activities in Wyoming:

- Wyoming Oil and Gas Conservation Commission
- Wyoming Department of Environmental Quality
- U.S. Bureau of Land Management
- U.S. Environmental Protection Agency.

The Oil and Gas Conservation Commission has the general authority over oil and gas production in the State. The Department of Environmental Quality is responsible for land applications of all types of exploration and production wastes. The Bureau of Land Management is responsible for all drilling and production on Federal lands.

The Wyoming Oil and Gas Conservation Commission does keep emissions inventories on accidental releases of $H_2S$. Any accidental release of the gas must be reported to the Commission immediately.

The enforcement, field monitoring, and inspection departments of the Oil and Gas Conservation Commission employ ten people. The Commission has the following enforcement options when compliance is not met: civil assessments, permits denial and revocations, and bond forfeiture.

In 1989, approximately 2,982 stripper wells in Wyoming produced over 5 million barrels of oil. In recent years, there have been no signs of noncompliance; however, there have been emergencies involving accidental $H_2S$ releases.

## FEDERAL REGULATORY PROGRAMS

Current Federal regulations potentially applicable to the oil and gas production industry's handling of hydrogen sulfide are summarized below. These include regulations of the Occupational Safety and Health Administration (OSHA), Bureau of Land Management, (BLM), U.S. Geological Survey, (USGS), Superfund Amendments and Reauthorization Act (SARA) Title III, the Clean Air Act, and others. Although the OSHA standards are applicable only to workers, they are analyzed as guidelines for reducing exposure to $H_2S$ from both accidental releases and routine emissions.

### OSHA Regulations

Currently, hydrogen sulfide emissions from oil and gas exploration and drilling are not directly addressed by OSHA regulations. The regulations that are in effect to protect workers are: OSHA Standards for General Industry (29 CFR Part 1910.1000), and the respirator standards (29 CFR Part 1910.134) and the OSHA Process Safety Management Standards (listed in Chapter III). Industries in which hydrogen sulfide occurs in quantities in excess of 1500 pounds are covered in the Process Safety Management of Highly Hazardous

Chemicals Standard (29 CFR 1910.119), but retail facilities and remote, unmanned operations are exempted. Oil and gas well drilling or servicing operations are also exempted. The potential exists that oil and gas operations that are the focus of this Report to Congress may be exempt from this OSHA standard if the facility is remotely located or if servicing operations include those studied in this Report. Table IV-6 lists current and proposed regulations pertaining to hydrogen sulfide.

Current Regulations

General Industry Standards (29 CFR 1910.1000). Acceptable concentrations for chemical exposure are listed in Section 1910 under Table Z-1-A., Limits for Air Contaminants, of the General Industry Standard (1910.1000). Effective December 31, 1992, the permissible exposure limit (PEL) time weighted average (TWA) for $H_2S$ is 1 x $10^4$ ppb) (14 mg/$m^3$). That is, an 8-hour time weighted average, such that an employee's exposure to hydrogen sulfide in any 8-hour workshift of a 40-hour workweek, shall not exceed 1 x $10^4$ ppb. Also for hydrogen sulfide, the short-term exposure limit (STEL) is 1.5 x $10^4$ ppb (21 mg/$m^3$). The 1.5 x $10^4$ ppb STEL is the employee's 15-minute (time weighted average) exposure, which shall not be exceeded at any time during the workday. The basis for the STEL is eye irritation.

The transitional OSHA standard, whose levels have been in effect since 1966, are ceiling limits and are listed in Table Z-2 of the OSHA standard. The acceptable ceiling concentration for hydrogen sulfide is 2 x $10^4$ ppb, with an acceptable maximum peak above the ceiling concentration of 5 x $10^4$ ppb lasting no more than 10 minutes, and occurring only once in an 8-hour shift, if no other measurable exposure occurs. The definition of a ceiling is the employee's exposure that shall not be exceeded during any part of the workday. If instantaneous monitoring is not feasible, then the ceiling shall be assessed as a 15-minute time weighted average exposure that shall not be exceeded at any time over a working day.

Respirator Standards (29 CFR 1910.134). The OSHA Personal Protective Equipment Standard (29 CFR 1910.134) outlines the types of personal protective devices (respirators) that should be worn when the ambient concentration exceeds the standards. Specific rules pertaining to hydrogen sulfide are not included in the standard. Covered in the standard are rules requiring written standard operating procedures, and employee training and screening for ability to use the equipment. Respirator selection, use, inspection and maintenance, storage, and cleaning are covered in the standard, as is air quality in supplied air respirators.

Process Safety Management of Highly Hazardous Chemicals (29 CFR 1910.119). The CAAA instructed OSHA (in section 304), in coordination with EPA, to promulgate a chemical process safety standard to prevent accidental releases of chemicals that could pose a threat to employees. This standard was finalized in February 1992 (57 *Federal Register* 6356).

The OSHA requirements for employers include standards to:

**Table IV-6. Summary of Occupational Exposure Standards for $H_2S$**

| Agency/Association | Background | Standard or Guideline |
|---|---|---|
| Occupational Safety and Health Administration (OSHA)[a]<br>General Industry Standards<br>29 CFR 1910.1000 | Current: Lists acceptable concentrations for chemical exposure in the work environment. $H_2S$ – listed under Table Z-1-A. | TWA 10 ppm 8-hour Time Weighted Average (TWA)<br>STEL 15 ppm 15-minute Short Term 29 Exposure Limit (STEL) |
| OSHA Respirator Standards<br>29 CFR 1910.134 | Current: Covers respirator selection, use, inspection and maintenance, storage and cleaning. Requires standard operating procedures; employee screening and training. | No specific rules pertaining to $H_2S$. |
| OSHA Process Safety Management of Highly Hazardous Chemicals Standards<br>29 CFR 1910.119 | Current: Remote unmanned facilities and drilling and servicing exempted.<br>Purpose: To prevent or minimize the consequences of catastrophic releases of highly hazardous chemicals. Some elements specified by the 1990 Clean Air Act Amendments. | Threshold quantity for $H_2S$: 1500 pounds; meaning that the potential exists for a catastrophic accident at facilities with more than 1500 pounds on site. |
| OSHA Oil and Gas Well Drilling and Servicing Standards<br>29 CFR 1910.270 | Proposed: 1983 proposal; OSHA still supports a specific standard for oil and gas production, thus their exemption from 29 CFR 1910.119 above. | Specifics pertaining to $H_2S$ include: monitoring programs, personal protective devices, automatic flare ignitors, spark arrestors, drilling mud programs. |
| National Institute for Occupational Safety and Health (NIOSH)[b]<br>Criteria Document for a Recommended Standard for Occupational Safety and Health | Recommendations for safe levels of worker exposure to $H_2S$.<br>Standards developed for healthy workers, not for the public at large. | $H_2S$ ceiling conc.: 15 mg/m$^3$ (approx. $1 \times 10^4$ ppb), 10-minute sampling, 10-hour workday, 40-hour workweek.<br>Evacuation: 70 mg/m$^3$ (approx. $5 \times 10^4$ ppb) |
| American Conference of Governmental Industrial Hygienists (ACGIH)[b]<br>Threshold Limit Values for Chemical Substances in the Work Environment | Professional organization of industrial hygienists which publishes annually updated Threshold Limit Values (TLVs) as guidelines in the control of ocupational health standards. | TLV-TWA: $1 \times 10^4$ ppb, for an 8-hour workday, 40-hour workweek.<br>TLV-STEL: $1.5 \times 10^4$ ppb, 15-minute weighted average, not more than 4 times/dayday. |

[a]Federal regulatory agency with enforceable standards; 25 of the States and territories run their own occupational safety programs.
[b]Recommended standard.

1) Develop and maintain written safety information identifying workplace chemical and process hazards, equipment, and process technology;

2) Perform a process hazard analysis which shall include an estimate of workplace effects of a range of releases and their health and safety effects on employees;

3) Consult with employees and their representatives on the conduct and development of the process safety management program.

4) Develop and implement written operating procedures for the chemical process;

5) Provide training to employees;

6) Evaluate and monitor contractor safety standards and performance;

7) Perform pre-startup safety reviews for new and modified facilities;

8) Establish maintenance systems for critical process related equipment;

9) Establish and implement written procedures to manage changes to the process;

10) Investigate every incident that has resulted or could result in a major accident;

11) Establish and implement a plant emergency action plan.

OSHA issued its final process safety standard on February 24, 1992.

Appendix A to the process safety standard (1910.119), lists the chemicals that present a potential for a catastrophic event with respective threshold quantities. For $H_2S$, the threshold quantity is 1500 pounds. This means that facilities with 1500 lbs or greater of $H_2S$ on-site would be subject to the process safety management standard. OSHA further requires that the 25 States and territories with their own occupational safety organizations adopt similar rules within 6 months.

Although hydrogen sulfide is covered in this standard, oil and gas drilling or servicing operations are exempted, along with retail facilities and normally unoccupied remote facilities. OSHA explains the reason for the drilling and servicing exemptions in its preamble to the final rulemaking (57 FR 6369), stating that "OSHA continues to believe that oil and gas well drilling and servicing operations should be covered in a standard designed to address the uniqueness of the industry." This exclusion is retained in the final standard since OSHA continues to believe that a separate standard dealing with such operation is necessary. The potential exists that oil and gas operations that are the focus of this Report to Congress may be exempt form this OSHA standard if the facility is remotely located or if servicing

operations include those studied in this Report. Table IV-6 lists current and proposed regulations pertaining to hydrogen sulfide.

Proposed Regulations

In 1983, OSHA proposed an Oil and Gas Well Drilling and Servicing Standard (48 FR 57202). The proposed standard would supplement the general standards already in effect and address the operation's unique hazards, such as those related to the unusual equipment, special situations dictated by the locations of operations, and hazards resulting from well pressures. According to the Bureau of Labor Statistics, the oil and gas well drilling and servicing industry was ranked among the most hazardous industries in the United States. OSHA estimated that 95,000 workers at approximately 5,400 rigs were employed in various occupations relating to oil and gas well drilling and servicing operations. The National Institute for Occupational Safety and Health (NIOSH) conducted a study of the oil and gas industry and provided OSHA with recommendations for developing a standard. In addition to a discussion of the Bureau of Labor Statistics injury data, NIOSH's "Comprehensive Safety Recommendation - Land Based Oil and Gas Well Drilling" also referenced in an early draft a study of data NIOSH received on fatalities and injuries occurring between 1973 and 1978 in Texas and California drilling operations. NIOSH applied these statistics for the entire drilling industry and concluded that the injury incidence and severity rates for the oil and gas drilling industry were more than six times the rate of general industry. However, these statistics include hazards other than $H_2S$.

In 1973 OSHA decided to regulate this industry under its Construction Safety Standards (29 CFR 1926); however, the applicability of this rule was contested by the industry. As a result of the industry contention, the Occupational Safety and Health Review Commission (OSHRC) ruled several times that the construction standards were not applicable. According to OSHRC, employers engaged in oil and gas well drilling and servicing should be subject to the general industry standards found in 29 CFR 1910. New enforcement problems emerged as a result of applying general industry standards. At the time of the issuance of the proposed standard, OSHA data showed that the oil and gas industry received a higher percentage of citations than any other industry. These citations are issued only when a standard does not exist to address the hazard, but the hazard is well recognized as a potential source of serious injury. OSHA felt that the high number of citations indicated the need for standards directed to these hazards in order to assist employers in meeting their obligations under the Occupational Safety and Health Act. They stated that it was apparent that the general industry standards either did not address or inadequately addressed hazards unique to oil and gas production, possibly even contributing to the higher injury and illness rate experienced by this industry. With the help of data from numerous studies of injury and illness in the oil and gas production industry, and input from numerous states, trade associations, labor unions and industry representatives, the draft oil and gas standards were proposed in 1983. No known action on this proposal has occurred since then. Currently, the proposed oil and gas well drilling and servicing rule has not been withdrawn, but it is also not on the regulatory agenda for finalizing.

OSHA proposed specific requirements for drilling, servicing, and special services operations performed in areas where a potential for exposure to $H_2S$ gas exists. The requirements proposed establishing and implementing a monitoring program in specified areas of the rig. The monitoring program would be applicable where the potential exists for $H_2S$ exposure, including areas where data are unavailable or inconclusive with respect to the potential $H_2S$ exposure. The program would use automatic environmental monitoring systems connected to an employee alarm system. Details of the program and its procedures would be required from the regulated community in written form. Testing and maintenance of the monitoring system would also be regulated under the proposal, because improperly maintained or untested systems may lead to a false sense of security for employees who rely on them for warning.

Specific respiratory protection equipment requirements were also included in the proposed regulation. All employees working in an area of potential hydrogen sulfide exposure would be required to wear or carry an approved escape-type, self-contained breathing apparatus. An approved positive-pressure respirator would be required for employees who remain in or return to the danger area.

In Appendix A to the proposed rule, OSHA also suggested the following practices to control or limit hydrogen sulfide exposure:

- automatic ignitors on the flare from the degasser, choke manifold, and mud-gas separator to burn off hydrogen sulfide;
- spark arrestors for all internal combustion engines to lessen the chance of the engine serving as a source of ignition in the event of a blowout;
- regular checking of drilling mud to assure it has the right constituents and pH to counteract $H_2S$;
- addition of hydrogen sulfide neutralizer to the drilling mud to prevent the gas from reaching the surface;
- installation of $H_2S$ monitoring systems on all rigs working within 1000 feet of known or suspected $H_2S$ zones.

Although the oil and gas well drilling and servicing rule (1910.270) was proposed in 1983 and has not been enacted, OSHA has continued to express a preference for a specific regulation pertaining to the oil and gas drilling and servicing operation in 1992, by exempting these industries from the Process Safety Management of Highly Hazardous Chemicals; Explosives and Blasting Agents Final Rule (29 CFR 1910.109 and 1910.119; 57 FR 6356).

Impact of OSHA Regulations on Occupational and Human Health

OSHA regulations are designed to protect the worker rather than the general public or the environment. In this respect, they set levels that protect the health of workers exposed for a 40-hour workweek, rather than residents who may be exposed continuously. The OSHA permissible exposure limit (PEL) for $H_2S$ is 10 ppb. Levels set to protect human health in general are often much more conservative since they are often based on models which assume exposure scenarios in which the person is exposed 24-hours a day for a lifetime. Non-occupational health effects levels may also account for possible developmental effects on young children and the effects of pollutants on those whose health is already compromised due to age or a chronic condition.

Four OSHA standards have the potential to protect workers exposed to $H_2S$. Two of these OSHA standards could apply to both workers and the public, while the other two apply specifically to workers. The OSHA general industry air contaminants and respirator standards protect the worker from $H_2S$ exposures above certain levels. These standards address the protection of the worker from an exposure in excess of a set level through the use of personal protective equipment. The public is not protected through these two standards, since they aim to protect workers from contact with $H_2S$ rather than prevent the release of the $H_2S$ into the atmosphere. The process safety management standard and the proposed oil and gas well drilling and servicing standard have the potential to protect both the worker and the general public by preventing the release of $H_2S$.

**National Institute for Occupational Safety and Health**

Recommendations for safe levels of worker environmental exposure to $H_2S$ are presented in the May 1977, *National Institute for Occupational Safety and Health (NIOSH) Criteria Document for a Recommended Standard for Occupational Exposure to Hydrogen Sulfide* (NIOSH, 1977). Hydrogen sulfide was cited as the leading cause of sudden death in the workplace (Ellenhorn and Barceloux, 1988). It was recognized as a serious hazard to the health of workers employed in energy production from hydrocarbon or geothermal sources, in the production of fibers or sheets from viscous syrup, in the production of deuterium oxide (heavy water), in tanneries, sewers, sewage treatment and animal waste disposal, in work below ground, fishing boats, and in chemical operations. Table IV-6 presents specific work practices recommended by NIOSH for the gas and oil industry.

A ceiling concentration was proposed to prevent eye effects and other adverse effects, including anorexia, nausea, weight loss, insomnia, fatigue, and headache, from prolonged exposure to hydrogen sulfide at low concentrations. The proposed ceiling concentration would also prevent acute eye effects, unconsciousness, and death, which can rapidly follow exposure to hydrogen sulfide at high concentrations. NIOSH suggests no employee be exposed to hydrogen sulfide at a ceiling concentration greater than 15 $mg/m^3$ (approximately $1 \times 10^4$ ppb), as determined with a sampling period of 10 minutes, for up to a 10-hour work shift in a 40-hour workweek. Evacuation of the area shall be required if the concentration of

hydrogen sulfide equals or exceeds 70 mg/m$^3$ (approximately 5 x 10$^4$ ppb). NIOSH warns that the standard was not developed for the population-at-large, and any extrapolation beyond occupational exposures is not warranted.

The document includes monitoring requirements for all areas where there is occupational exposure to $H_2S$. First, there should be personal monitoring to detect each employee's ceiling exposure, with source and area monitoring as a supplement. The monitoring should be done quarterly, or as recommended by an industrial hygienist. Recording automatic monitors would be permitted to show short-term (less than 1-minute) peaks of up to 5 x 10$^4$ ppb, as long as no more than one occurs in any 30-minute period. These recording automatic monitors should be set up to signal spark-proof audible or visual alarms. They should have different alarms to signal concentrations of 1 x 10$^4$ ppb as an alert level to employees and 5 x 10$^4$ ppb as the level for employee evacuation.

The Secretary of Labor weighs NIOSH's recommendations, along with other considerations such as feasibility and means of implementation, in developing regulatory standards. The criteria document also contains sections on medical screening and followup of exposed employees, labeling and posting of $H_2S$ hazards, personal protective equipment, hazard information for employees, work practices, sanitation, and monitoring and recordkeeping.

**Bureau of Land Management**

If a sour oil and gas well is located on Federal or Indian land, the facility operator or owner is subject to the requirements imposed by the Onshore Oil and Gas Order No. 6 developed by the Bureau of Land Management. This order requires submittal of a public protection plan by operators of sour oil and gas facilities upon detection of the potential to release a hazardous volume of $H_2S$ (defined as concentrations of $H_2S$ that exceed 1 x 10$^5$ parts per billion in the gas stream). Site-specific conditions are also criteria for determining whether or not a facility needs to submit a public protection plan. These conditions include (1) proximity to public buildings, public gathering centers, and roadways used for public use; and (2) radius and concentration of exposure. The order also has requirements for danger signs, fencing and gates, and wind direction indicators. Additional requirements include well control equipment, corrosion protection, and automatic safety valves or shutdowns for accidental release prevention.

The Bureau of Land Management does have procedures for enforcing Onshore Oil and Gas Order No. 6. Penalties for failure to comply with are cited in 43 C.F.R. 3163.1 (1992).

**Minerals Management Service**

The Minerals Management Service (Department of the Interior) Outer Continental Shelf Standard, MMS-OCS-1, Safety Requirements for Drilling Operations in a $H_2S$

Environment is the name for the former U.S. Geological Survey Outer Continental Shelf (OCS) Standard No.1. In February of 1976, the Conservation Division of the U. S. Geological Survey (USGS) released offshore rules for safety and pollution prevention in Standard No. 1, Safety Requirements for Drilling Operations in a Hydrogen Sulfide Environment (USGS, 1976). Required details of a contingency plan for emergency hydrogen sulfide situations are listed in the standard, and each platform is required to have the plan developed prior to drilling. The standard also specifies details of the personnel training program, and type, storage location and use of personnel protective equipment. Finally, the standard requires state-of-the-art equipment for blowout prevention, and specifies details of the mud program, well-testing procedures and flare system.

The standard requires $H_2S$ monitoring equipment at all wells, except when drilling in areas known to be free of hydrogen sulfide. Upon encountering hydrogen sulfide, the safety requirements of the rules go into effect, and when concentrations reach $2 \times 10^4$ ppb the remainder of the rules dealing with hydrogen sulfide's corrosive effects must be observed. The precautions in the American Petroleum Institute Recommended Practice for Safe Drilling of Wells Containing Hydrogen Sulfide, (API RP 49) are considered supplemental to the requirements of the standard (API, 1987).

Two separate operational conditions are outlined with requirements for warning flags and notification of authorities. Moderate danger, when the threshold limit value of 10 ppm is reached, requires the display of signs and flags reading "DANGER - HYDROGEN SULFIDE - $H_2S$." If the concentration reaches $2 \times 10^4$ ppb, protective-breathing apparatus is required to be worn by all working personnel, and non-working personnel are required to evacuate to safe briefing areas. Extreme danger, when $H_2S$ reaches the injurious level ($5 \times 10^4$ ppb), is the point when all personnel (or all non-working personnel as appropriate) are required to evacuate. Radio communications are required to alert all known air and water craft in the immediate vicinity of the danger.

The Minerals Management Service is in the process of reproposing its standards for hydrogen sulfide.

**CERCLA and EPCRA**

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980 establishes broad Federal authority to deal with releases or threatened releases of hazardous substances from vessels and facilities. The Act defines a set of hazardous substances chiefly by reference to other environmental statutes; currently there are over 700 CERCLA hazardous substances. Commonly known as "Superfund," CERCLA requires that the person in charge of a vessel or facility notify the National Response Center as soon as that person has knowledge of a release of a hazardous substance in an amount equal to or greater than the reportable quantity (RQ) for that substance. Currently, hydrogen sulfide is listed as a CERCLA hazardous substance with a reportable quantity of 100 pounds.

On October 17, 1986, the President signed into law the Superfund Amendments and Reauthorization Act of 1986 (SARA), which revises and extends the authorities established under CERCLA and other laws. The Emergency Planning and Community Right-to-Know Act (EPCRA), enacted in 1986 as Title III of SARA, establishes new authorities for emergency planning and preparedness, community right-to-know reporting, and toxic chemical release reporting. It is intended to encourage and support emergency planning efforts at the State and local levels and to provide citizens and local governments with information concerning potential chemical hazards present in their communities. EPCRA is organized into three subtitles (A-C), each containing a number of subsections.

Subtitle A establishes the framework for State and local emergency planning. Section 301 requires each State to establish an emergency response commission and local emergency planning committees. Section 303 governs the development of comprehensive emergency response plans by local emergency planning committees and provision of facility information to the committee. Section 302 requires EPA to publish a list of extremely hazardous substances and threshold planning quantities (TPQs) for such substances. This list was established by EPA to identify chemical substances that could cause serious irreversible health effects from accidental releases. The list includes hydrogen sulfide, with a threshold planning quantity of 500 pounds. Any facility where an extremely hazardous substance is present in an amount in excess of the threshold planning quantity is required to notify the State commission and be included in local planning efforts. Section 304 establishes requirements for immediate reporting of certain releases of reportable quantities of extremely hazardous substances, and CERCLA Hazardous Substances, to the local planning committees and State emergency response commissions. These requirements are similar to the release reporting provisions under Section 103 of CERCLA. Section 304 also requires follow-up reports on each release, its effects, and response actions taken.

Only those sour oil and gas wells and well-site facilities that have 500 pounds or more of $H_2S$ present at the well facility are subject to the planning requirements. The reportable quantity of $H_2S$ is 100 pounds. Therefore, releases into the environment at or above 100 pounds must be reported in accordance with CERCLA 103 and EPCRA 304.

Subtitle B provides the mechanism for community awareness of hazardous chemicals present in the locality. This information is critical for effective local contingency planning. If the owner or operator of a facility is required to prepare or have available a Material Safety Data Sheet (MSDS) for a hazardous chemical under the Occupational Safety and Health Act of 1970 and regulations promulgated under that Act, Section 311 requires that owner or operator to submit MSDSs, or a list of the chemicals for which the facility is required to have an MSDS, to the local emergency planning committees, State emergency response commissions, and local fire departments. Under Section 312, owners and operators of facilities that must submit an MSDS under Section 311 are also required to submit chemical inventory information on the hazardous chemicals present at the facility. The threshold for reporting for $H_2S$ under sections 311 and 312 is 500 pounds. Only facilities that have more than the threshold quantity need to report under sections 311 and 312, unless

MSDS or inventory information is specifically requested by the State Emergency Response Commission (SERC) or Local Emergency Planning Committee (LEPC). The owner or operator must submit an inventory form containing an estimate of the maximum amount of hazardous chemicals present at the facility during the preceding year, an estimate of the average daily amount of hazardous chemicals at the facility, and the location of these chemicals at the facility. Section 313 requires that certain facilities with ten or more employees that manufacture, process, or use a "toxic chemical" in excess of a statutorily-prescribed quantity submit annual information on the chemical and releases of the chemical into the environment. This information must be submitted to EPA and to the appropriate State offices annually. Hydrogen sulfide is not listed as a toxic chemical for which annual release information is required.

Subtitle C contains general provisions concerning trade secret protection, enforcement, citizen suits, and public availability of information.

**Clean Air Act Section 112(r) - Accident Prevention**

The Clean Air Act Amendments of 1990 established programs to prevent accidental releases of extremely hazardous substances and to assure that mitigation and response measures are in place in the event that a release does occur. Section 112(r) of the Clean Air Act establishes the responsibility for prevention of releases of extremely hazardous substances as the general duty of owners and operators of facilities that produce, process, handle or store such substances. Section 112(r) also requires that EPA promulgate a list of at least 100 substances that could cause death, injury or serious adverse effects to human health or the environment. Facilities with threshold quantities of the listed substances will be required to establish risk management programs and to prepare risk management plans. The statute requires EPA to promulgate regulations concerning risk management plans and other aspects of accident prevention. $H_2S$ is one substance to which these requirements will apply as mandated in the statute.

The general duty clause is intended to establish as a responsibility of the facility owner the prevention of accidental releases and minimization of the consequences of accidental releases which do occur. Responsibilities include the conduct of appropriate hazard assessments and the design, operation, and maintenance of a safe facility. This means that facilities must be equipped for release mitigation and community protection should a release occur. The clause in the Clean Air Act Amendments refers to and is correlated with the general duty clause contained in the Occupational Safety and Health Act administered by OSHA. The OSHA clause was designed for situations for which there is no specific OSHA regulation or standard. Recognition of the hazard by the owner or operator, or within an industry, of the industry has been one standard under the OSHA general duty clause (U.S. Senate 1989). Therefore, the general duty clause places on the owners and operators of facilities the responsibility to adhere to applicable industry codes and standards for safety, accident prevention, and response.

The accidental release prevention list criteria include severity of acute adverse health effects, likelihood of accidental release, and potential magnitude of human exposure. A threshold quantity is to be established for each regulated substance to account for toxicity, dispersibility, reactivity, volatility, combustibility, or flammability of the substance and the amount anticipated to cause adverse health effects in an accidental release. The list and threshold quantities were proposed on January 19, 1993 (58 FR 5102). $H_2S$ is listed as a toxic, and other substances present at oil and gas sites, such as methane, ethane, propane, and other hydrocarbons, are listed as flammables. Facilities with threshold quantities of the regulated substances will be required to prepare risk management plans (RMPs) and implement risk management programs. The RMPs will include a summary of assessments of offsite consequences for a range of accidental releases (including worst-case accidental releases) and a history of accidental releases. Facilities must also describe release prevention and emergency response programs developed under the risk management regulations as part of the RMP process.

**Clean Air Act - PSD Program**

There is no NAAQS which addresses hydrogen sulfide; however, emissions of $H_2S$ are regulated under the Prevention of Significant Air Quality Deterioration (PSD) Program. PSD is designed to allow for industrial growth within specific air quality goals. The basic goals of the PSD regulations are (1) to ensure that economic growth will occur in harmony with the preservation of existing clean air resources to prevent any new nonattainment problems; (2) to protect the public health and welfare from any adverse effect which might occur even at air pollution levels better than the national ambient air quality standards; and (3) to preserve, protect and enhance the air quality in areas of special national or regional natural, recreational, scenic, or historic value, such as national parks and wilderness areas.

PSD permits are required for stationary sources located in areas designated, pursuant to section 107 of the CAA, as attainment or unclassifiable for a criteria pollutant. Major sources or modifications are those emitting either at least 100 tons per year or 250 tons per year of any pollutant regulated under the CAA, depending on the source category of the PSD listed pollutants. Major sources in nonattainment areas would be regulated under permit requirements persuant to Part D under title I of the CAA.

The CAA has set significance levels, below which a PSD permit is not required. Two tables set the significance values, one for defining significant emissions changes, in tons per year; and the other for defining significant air quality changes, in $\mu g/m^3$. For hydrogen sulfide, the applicable emissions threshold is the significant emission rate of 10 tons per year. An exemption from the monitoring provision of the permitting regulations for hydrogen sulfide is set as a 1-hour average concentration of 0.02 $\mu g/m^3$. Hydrogen sulfide emissions are also counted as part of the Total Reduced Sulfur and Reduced Sulfur, both having significance values set at 10 tons per year. These pollutant classes are regulated primarily to avoid nuisance (odor) problems.

The applicability of the PSD permit program to oil and gas extraction wells would be dependent on the amount of emissions and the grouping of the wells (i.e., whether several wells would be combined for calculation of emissions). In general, it appears that most oil and gas extraction wells would not likely be subject to PSD regulations based on the applicability criteria.

## INDUSTRY-RECOMMENDED SAFETY AND ENVIRONMENTAL PROTECTION PROCEDURES

This section summarizes selected industry standards and practices for managing $H_2S$ releases to the atmosphere. The American Petroleum Institute (API) has developed and published design, construction, and operating standards. Certain aspects of these standards pertaining to accidental release prevention were discussed in the previous chapter.

### API Recommended Practices

The American Petroleum Institute (API), an industry-wide technical organization, has published several recommended practices (RP) pertaining to hydrogen sulfide in the oil and gas production industry. These voluntary guidelines are intended to maintain worker and public safety and health. Table IV-7 lists API Recommended Practices pertinent to production and operations in formations containing $H_2S$.

#### Control Standards

API RP 49, Recommended Practices for Safe Drilling of Wells Containing Hydrogen Sulfide (April 15, 1987) and API RP 55, Recommended Practices for Conducting Oil and Gas Production Operations Involving Hydrogen Sulfide (October 1981; reissued March, 1983; and preparation of a second edition began in 1990) are the two main documents dealing with $H_2S$ in oil and gas production. It is expected that the revised RP 55 will provide information similar in scope to that in the document currently under revision, but with additional detail and more current references. These recommended practices do not set a control level for $H_2S$ emissions; rather they identify situations to which the practices apply. They are applicable in oil and gas operations where the potential exists for atmospheric concentrations of $H_2S$ to reach $2 \times 10^4$ ppb. They also apply "where the fluids handled contain sufficient $H_2S$ to produce a partial pressure above 0.05 pounds per square inch absolute (psia) and the total pressure is 65 psia or greater, or where internal or external stresses are present which could result in pipe or equipment failure due to sulfide stress cracking and/or hydrogen embrittlement" (API, 1987). In these cases, materials must meet National Association of Corrosion Engineers (NACE) standards.

#### Control Techniques

The control techniques discussed in the API Recommended Practices take two approaches to worker and public safety. First, when hydrogen sulfide has already been

**Table IV-7. Reviewed American Petroleum Institute* Documents Pertaining to $H_2S$ in Oil and Gas Production**

| Document | Date | Title | Topics Covered |
|---|---|---|---|
| Recommended Practice 49 (RP49) | 2nd Edition April 15, 1987 | Recommended Practices for Safe Drilling of Wells Containing Hydrogen Sulfide | Personnel training and protective equipment. Locations. Rig and well equipment. Rig operations in $H_2S$ environments. Contingency planning and emergency procedures. Properties and effects of $H_2S$ and $SO_2$. Sour environment definition. |
| Recommended Practice 51 (RP51) | 1st Edition October 1974 Reissued May 1982 | API Recommended Onshore Production Operating Practices for Protection of the Environment | Producing wells. Lease roads, gathering systems and pipelines. Production and water handling facilities. Oil discharge – prevention and cleanup. |
| Recommended Practice 53 (RP53) | 2nd Edition May 25, 1984 | Recommended Practice for Blowout Prevention Equipment Systems for Drilling Wells | Arrangement (surface and subsea) and/or installation of: blowout preventers, choke and kill units and lines, closing units, auxilliary equipment, pipe stripping, marine riser systems. Inspection and testing. Sealing components. Blowout modifications for $H_2S$ environments. |
| Recommended Practice 54 (RP54) | 2nd Edition May 1, 1992 | Recommended Practices for Occupational Safety for Oil and Gas Well Drilling and Servicing Operations | Injuries and first aid. Protective equipment. Fire prevention. Drilling and well servicing rig equipment and electrical systems. Wireline service. Stripping and snubbing. Drill stem testing. Operations (including $H_2S$ environment). |
| Recommended Practice 55 (RP55) | 1st Edition October 1981 Reissued March 1983 (revision in progress) | Recommended Practices for Conducting Oil and Gas Production Operations Involving Hydrogen Sulfide | Personnel training and protective equipment. Contingency plans and emergency procedures. Design, construction, and operating procedures. Surveillance and maintenance. Continuous $H_2S$ monitoring equipment. Supplementary guidance and reference material for $H_2S$ operations. |
| Specification 6A (SPEC 6A) | 16th Edition October 1, 1989 | Specification for Wellhead and Christmas Tree Equipment, Supplement 1 and 2 | Design and performance. Materials. Welding. Quality control. Equipment marking, shipping, storing, and specific requirements. |

*American Petroleum Institute; 1220 L Street, Northwest; Washington, DC 20005.

released, worker and public safety is protected through the use of monitoring programs, personal protective devices and contingency plans for evacuations. Second, the engineering approach uses design, construction, and operating procedures to prevent the release of hydrogen sulfide to the atmosphere. The prevention of equipment damage due to corrosion (sulfide stress cracking) and the techniques for prevention of blowouts in API RP 53, Recommended Practice for Blowout Prevention Equipment Systems for Drilling Wells, are two main considerations in this more site- specific engineering control technique.

API RP 49, which deals with drilling in a hydrogen sulfide environment, contains the following recommendations for well siting in order to protect workers from the effects of hydrogen sulfide accumulation at the well site: "Rig components should be arranged on a location such that prevailing winds blow across the rig in a direction that will disperse any vented gas from the areas of the wellhead, choke manifold, flare stack or line, mud/gas separator, drilling fluid tanks, reserve pits, shale shaker, and degasser away from any potential ignition source (i.e., engines, generators, compressors, crew quarters, etc.) and areas used for personnel assembly. All equipment should be located and spaced to take advantage of prevailing winds and to provide for good air movement to eliminate as many sources of potential gas accumulation as possible" (API, 1987).

Other siting recommendations in API RP 49, shown in Figure III-4, are the use of caution signs at entrance and exit roads to warn of hydrogen sulfide concentrations above $2 \times 10^4$ ppb and danger flags to warn of extreme danger when the concentration exceeds $5 \times 10^4$ ppb. These signs are required to stay in place when flaring of the hydrogen sulfide could produce sulfur dioxide concentrations in excess of $5 \times 10^3$ ppb. Protection or briefing centers should be placed upwind or perpendicular to the prevailing wind, with wind direction indicators easily visible from the briefing location and all work locations. Mechanical ventilation, large fans or bug blowers, should be available for use during light wind conditions to prevent the hydrogen sulfide from accumulating in low lying locations. The locations of drilling fluid systems, power plants, burn pits, and flare stacks are also discussed from the vantage point of worker safety after the release of hydrogen sulfide.

Both API RP 49 (pertaining to drilling in a hydrogen sulfide environment) and API RP 55 (dealing with production operations) contain recommendations for personnel training. RP 55 training program topics include: the effects upon humans of various concentrations of hydrogen sulfide; protective equipment, including the use of self contained breathing apparatus rather than canister type gas masks (a filtering type mask is not appropriate for protection from hydrogen sulfide); monitoring devices; emergency procedures; material selection; and the importance of ventilation. Monitoring equipment that would set off a visual alarm at $1 \times 10^4$ ppb and an audible one at $2 \times 10^4$ ppb is recommended. Breathing equipment requirements are also discussed, including selection and storage (where they are readily available in an emergency).

Contingency plans are outlined in Section 4 of API RP 55 (API, 1983). They are recommended for each operation that has the potential for an accidental release capable of

exposing the public to hazardous concentrations of hydrogen sulfide. Contingency plans should include the locations of: equipment that contains hydrogen sulfide, residences and other public facilities, evacuation routes, safety equipment, telephones, and designated briefing areas for employees. The contingency plan should also include procedures for calculating the dispersion of releases and lists of emergency telephone numbers. Finally, it is suggested that public and local officials should be briefed about the potential hazard prior to an incident, and that periodic tests of the contingency plan should be conducted.

RP 55 also covers protection of workers from the toxic effects of hydrogen sulfide due to build-up of gas concentration in confined areas. Protective equipment or purging is recommended for vessels that have previously held hydrogen sulfide. Extreme caution should be used when entering buildings containing equipment used to handle fluids containing hazardous concentrations of hydrogen sulfide. Routine use of personal protective devices is suggested in these instances.

API RP 54, Recommended Practices for Occupational Safety for Oil and Gas Well Drilling and Servicing Operations (May 1, 1992) also addresses some aspects of personal protection from the toxic effects of hydrogen sulfide (API, 1992). This document was released after OSHA's implementation of the $1 \times 10^4$ ppb time-weighted average standard. RP 54 does not mention any specific standard or level, rather it refers the reader back to API RP 49 and API RP 55, which state that they apply to oil and gas operations where the potential exists for atmospheric concentrations to reach $2 \times 10^4$ ppb (the old OSHA ceiling standard), or where the gas could cause corrosion of the equipment. API does caution throughout their documents that the latest local, State and Federal regulations should be consulted.

Engineering controls used to prevent the production of, or the release of, hydrogen sulfide to the atmosphere are covered in the recommended practices for drilling and production (RP 49 and RP 55). API RP 55, pertaining to production, warns of the potential for introducing sulfur-reducing bacteria, which produce hydrogen sulfide, into a formation during pressure maintenance or water flooding operations (i.e., enhanced oil recovery). Operators are warned to be aware of the possibility and to act quickly if introduction occurs. If care is taken to prevent the bacteria from being introduced into formations that do not contain hydrogen sulfide, the danger of hydrogen sulfide pollution will be prevented.

Other engineering controls such as those used in design, construction, and operating practices are covered in Section 5 of RP 55. API recommends that construction materials meet specifications of the National Association of Corrosion Engineers (NACE) Standard MR-01-75: Material Requirements for Sulfide Stress Cracking Resistant Metallic Material for Oil Field Equipment. These materials include all those that are exposed to fluids containing hydrogen sulfide and critical to its containment. Process factors for consideration are discussed, including the concentration of hydrogen sulfide, the maximum atmospheric temperatures expected, pressure, pH, water content of fluids, mechanical stresses, corrosional or scale effects on the system, and any others unique to each situation. Finally,

piping design should eliminate dead or slow-flow areas where fluids containing hydrogen sulfide gas can collect.

Drilling fluids are important to the control of the drilling environment. According to API RP 49 (Recommended Practices for Safe Drilling of Wells Containing Hydrogen Sulfide), the following practices help to maintain environmental control: maintenance of a pH of 10 or higher to neutralize hydrogen sulfide (failing to maintain proper pH can cause release of hydrogen sulfide from the drilling fluid system), the use of chemical sulfide scavengers, and the use of oil-based drilling fluids. When hydrogen sulfide gas is breaking out of drilling fluids, the fluids should be routed through a mud-gas separator until the level is reduced to a safe one. Corrosion inhibitors that create a film which protects the equipment from pitting and eventual sulfide stress cracking are also recommended. Finally, extreme caution is urged in storing fluids that have been exposed to hydrogen sulfide, and in entering enclosed areas where drilling fluids have been stored.

Drill stem, casing, tubing, and wellhead selection must meet specifications of API, NACE, the American Society of Mechanical Engineers, and the American National Standards Institute, detailed in Section 5 of RP 49. Section 5 also covers procedures for limited entry tests and equipment considerations for blowout preventer units, closing units, remote choke control lines, and kill lines. Hydrogen sulfide considerations in mud/gas separators, degassers and flare system are also discussed.

Abandonment procedures are included in API RP 55, with the disclaimer that the suggested procedures do not supersede local, State or Federal regulations. Section 6.5 discusses spontaneous combustion of iron sulfide, which is produced by the reaction of $H_2S$ with steel. Because spontaneous combustion is possible when iron sulfide is exposed to air, RP 55 suggests that iron sulfide be kept wet until it can be burned or buried. Iron sulfide also poses a hazard during well servicing operations. Acids react with the iron sulfide to produce $H_2S$. Damage may also occur in pipes exposed alternately to hydrogen sulfide and air. API stresses the use of monitoring equipment when well servicing operations are performed on wells where a hydrogen sulfide hazard exists.

Hydrogen sulfide in oil and gas production is also mentioned in API RP 51, API Recommended Onshore Production Operating Practices for Protection of the Environment (October 1974, reissued May 1982). General information on the protection of personnel and equipment are presented in this document (API, 1982).

## FINDINGS

1. Eighteen States have short-term $H_2S$ ambient air quality standards. Four of the nine major oil and gas producing States reviewed in this report do not have ambient air standards.

2. Ambient air quality standards range from 160 ppb per 24 hr average time to 50 ppb per 0.5 hr average time.

3. The number of State agencies involved in controlling oil and gas operations varies widely.

4. The size of enforcement staffs at the State level varies greatly, with some staff having inspection responsibility beyond oil and gas operations.

5. No specific $H_2S$ environmental (i.e., ecological) protection standards were found for Texas, Michigan, Oklahoma and California.

6. Not all States maintain notification requirements for accidental releases of $H_2S$ from oil and gas wells. Some do require notification when a threatening accidental release occur.

7. Reporting of routine $H_2S$ emissions is not required in Texas, Oklahoma, Michigan, or California. "Routine" excludes such incidents as vapor recovery unit failures and other equipment upsets.

8. NIOSH suggests no employee be exposed to $H_2S$ at a ceiling concentration greater than 15 $mg/m^3$ (about $1 \times 10^4$ ppb) for up to a 10 hr work shift in a 40 hr work week. Evacuation is required if the concentration equals or exceeds 70 $mg/m^3$ ($5 \times 10^4$ ppb).

9. NIOSH requires monitoring in work areas with alarms sounding at $1 \times 10^4$ ppb and $5 \times 10^4$ ppb.

10. The Minerals Management Service requires for offshore rigs drilling in an $H_2S$ environment: contingency plan, personnel training, state-of-the-art blowout prevention equipment, monitoring equipment and response procedures at $1 \times 10^4$, $2 \times 10^4$, and $5 \times 10^4$ ppb. Special mud programs, well-testing procedures, and flare systems are also required. This Federal regulatory program does not have an equivalent onshore program.

11. The PSD permit program applies to significant emissions of $H_2S$ from new sources emitting greater than 250 tons per year (or 100 tons per year for certain source categories) of any regulated pollutant, i.e., major PSD sources. It also applies to modifications of existing facilities if the net emissions increase of $H_2S$ from the modification is significant. In either case, the significant emission rate for $H_2S$ is 10 tons per year. Also, permits do not require monitoring if the 1-hr average concentration is below 0.014 ppb (0.02 $\mu g/m^3$). $H_2S$ is also regulated under the PSD program for its nuisance odor as part of a larger group of Total Reduced Sulfur and Reduced Sulfur (significant $\geq$ 10 tons/yr).

12. Accidental releases of $H_2S$ can be prevented by application of process safety management principles. The following are among the ways that these principles are adopted:

   a. Under the Clean Air Act, as amended, industry has a responsibility to identify hazards, take the actions necessary to prevent chemical accidents, and to take action to mitigate accidents in the event that they do occur.
   b. OSHA has promulgated a process safety management standard that requires facilities to implement process safety management programs for chemicals including $H_2S$ to protect workers from accidents. These same measures can also prevent chemical accidents that might affect the public. However, the OSHA requirements do not apply to remote or unstaffed facilities such as most oil and gas well sites.
   c. Under the Clean Air Act, as amended, EPA must promulgate rules that require facilities handling $H_2S$ to implement a risk management plan designed to prevent chemical accidents that adversely affect the public.
   d. The Bureau of Land Management's Onshore Oil and Gas Order No. 6 addresses the prevention of accidental releases of $H_2S$ on Federal or Indian lands.
   e. Several State programs address the prevention of accidental releases of $H_2S$. States with such programs include Oklahoma, Texas, Michigan, California, and New Mexico.
   f. Voluntary industry initiatives (e.g., codes, standards, recommended practices) such as the API RP 55, Recommended Practices for Conducting Oil and Gas Operations Involving $H_2S$, which is currently being revised, have been implemented by many facilities.

13. A number of Federal and State requirements exist for emergency planning in the event that an accidental release of $H_2S$ occurs.

   a. Facilities handling quantities of $H_2S$ greater than threshold amounts are subject to the emergency planning requirements of the Emergency Planning and Community Right-to-Know Act (EPCRA).
   b. The accidental release prevention provisions of the Clean Air Act Amendments will require facilities handling amounts of $H_2S$ above threshold quantities to implement an emergency response program.
   c. For Federal and Indian lands, the Bureau of Land Management requires public protection plans for sour oil and gas production operations that meet certain criteria.
   d. Several States require contingency plans in the event of accidental $H_2S$ releases. State requirements include those of Oklahoma, Texas, Michigan, California, and New Mexico.

e. API RP 55 recommends that contingency plans be developed for oil and gas extraction facilities where an accidental release of $H_2S$ could be immediately hazardous to life or health.

## REFERENCES


API. 1982. *API Recommended Practice 51 (RP 51), Recommended Onshore Production Operating Practices for Protection of the Environment*, October 1974, Reissued May 41982, 1st ed., Publication No. RP51. American Petroleum Institute.

API. 1983. *API Recommended Practice 55 (RP 55), Recommended Practices for Conducting Oil and Gas Production Operations Involving Hydrogen Sulfide*, October 1981, Reissued March 1983, 1st ed., Publication No. RP55. American Petroleum Institute.

API. 1984. *API Recommended Practice 53 (RP 53), Recommended Practice for Blowout Prevention Equipment Systems for Drilling Wells*, 2nd ed., Publication No. RP53. American Petroleum Institute.

API. 1987. *API Recommended Practice 49 (RP 49), Recommended Practices for Safe Drilling of Wells Containing Hydrogen Sulfide*, 2nd ed., Publication No. RP49. American Petroleum Institute.

API. 1989. *API Specification 6A (SPEC 6A), Specification for Wellhead and Christmas Tree Equipment*, with Supplement 1 and 2, 16th ed., Publication No. SPEC6A. American Petroleum Institute.

API. 1992. *API Recommended Practice 54 (RP 54), Recommended Practices for Occupational Safety for Oil and Gas Well Drilling and Servicing Operations*, 2nd ed., Publication No. RP54. American Petroleum Institute.

California Air Resources Board. 1991. Multicounty Air Pollution Control District Map. Sacramento, California.

CDC. 1991. *California Laws for Conservation of Petroleum and Gas*. Publication No, PRC01. California Department of Conservation.

Dosch, M.W., and Hodgson, S.F. 1986. *Drilling and Operating Oil, Gas, and Geothermal Wells in an $H_2S$ Environment*, Publication No. M10. California Department of Conservation, Division of Oil and Gas, Sacramento, CA.

Ellenhorn, M.J., and Barceloux, D.G. 1988. *Medical Toxicology*. Elsevier Science Publishing Co., New York, NY.

*Guidelines for Petroleum Emergency Field Situations in the State of Oklahoma.* 1983. Recommended to the Oklahoma Petroleum Industries by the Industry Advisory Committee and the Oklahoma Corporation Commission - Oil and Gas Corporation Conservation Division. October 1, 1983.

IOGCC. 1990. *The Interstate Oil and Gas Compact Committee Bulletin,* Volume IV, Number 2. The Oil and Gas Compact Commission, Dallas, TX.

Michigan's Oil and Gas Regulations - Act 61 (P.A. 1939 as amended and promulgated rules - Circular No. 15, revised in 1987). Michigan Department of Natural Resources.

NIOSH. 1977. *NIOSH Criteria for a Recommended Standard.... Occupational Exposure to Hydrogen Sulfide*, Publication No. 77-158. U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health, Cincinnati, OH, 2, 147.

OCC. 1986. The Corporation Commission of the State of Oklahoma. General Rules and Regulations of the Oil and Gas Conservation Division.

*Oil and Gas Operators' Manual.* Pennsylvania.

Petroleum Independent. 1992. The Oil and Natural Gas Producing Industry in Your State, 1992-1993. *Petroleum Independent*, Vol. 62. No. 7. Independent Petroleum Association of America.

*Statewide Order Governing the Drilling for and Producing of Oil and Gas in the State of Louisiana.*

U.S. Geologic Survey. 1976. *Outer Continental Shelf Standard No. 1, Safety Requirements for Drilling in a Hydrogen Sulfide Environment*, GSS-OCS-1.

U.S. Senate. 1989. Report on The Clean Air Act Amendments of 1989, S-1630. U.S. Government Printing Office.

# CHAPTER V
# RECOMMENDATIONS

## ROUTINE EMISSIONS

From the limited data available, there appears to be no evidence that a significant threat to public health or the environment exists from routine $H_2S$ emissions from sour oil and gas extraction. States and industry are encouraged to evaluate existing design, construction, and operation principles within the framework of process safety management. EPA recommends no further legislation pertaining to routine $H_2S$ emissions from oil and gas extraction at this time.

## ACCIDENTAL RELEASES

### General

The EPA recommends no further legislative action pertaining to accidental $H_2S$ releases from oil and gas extraction activities at this time. The regulations already promulgated, and being developed, under the authorities provided to EPA in CERCLA, EPCRA, and the accidental release prevention provisions of the CAA, provide a good framework for the prevention of accidental releases and preparedness in the event that they occur.

- EPA should track implementation of current and future industry standards and recommended practices at sour oil and gas extraction facilities. An example of such industry standards is the American Petroleum Institute Recommended Practices for Conducting Oil and Gas Production Operations Involving Hydrogen Sulfide (API RP55). EPA should consider outreach specifically directed at non-participating sectors.

- The EPA should participate in the investigation of any accidental releases associated with $H_2S$ that cause or have the potential to cause public impacts in order to determine the root cause of such accidents. Such investigations should be coordinated with the Occupational Safety and Health Administration (OSHA) in order to encompass worker safety issues.

- The EPA should continue to investigate the need for additional rulemaking under the accidental release prevention provisions of the Clean Air Act to require implementation of certain prevention, detection, monitoring and mitigation efforts at facilities where extremely hazardous substances (such as $H_2S$) could generate dense gas clouds and impact the public. The level of voluntary industry initiatives and degree of participation, and accident history should be taken into account.

**Facility and Local Emergency Planning Committee (LEPC)**

Facilities that handle hazardous substances that could form dense vapor clouds if accidentally released, such as $H_2S$, should work closely with their LEPC to prevent accidents and to be prepared to respond to such accidents.

- Facilities should identify and thoroughly understand the hazards and conditions that can lead to accidental releases and the potential impacts on the public. These hazards and potential impacts should be communicated to the LEPC.

- All sour oil and gas extraction facilities and the LEPC for that area should conduct drills and exercises with workers, the community, first responders and others to test mitigation, response, and medical treatment for a simulated major $H_2S$ accident. All such facilities should have training programs in place for $H_2S$ emergencies.

**Preparedness and Response**

All sour oil and gas extraction facilities should actively conduct outreach efforts to ensure that the community is aware of the hazards of $H_2S$, that protective measures are in place to prevent public health impacts, and that proper actions will be taken during an emergency. Such outreach should be conducted through the LEPCs.

- All sour oil and gas extraction facilities should able to rapidly detect, mitigate, and respond to accidental releases in order to minimize the consequences. Site-specific risk factors should be taken into account.

- Because a general duty exists to design, operate, and maintain a safe facility, owners and operators of sour oil and gas facilities should use appropriate equipment for the facility to provide public safety and should implement a program to remedy the effects of wear and tear and corrosion on equipment.

- In addition to regular inspection of all equipment, owners and operators should pay particular attention to corrosion monitoring of existing flow and gathering lines and to the condition of temporarily abandoned equipment. Remedial action should be taken before accidental releases occur.

- EPA should foster the development and continued refinement of release detection and mitigation systems for hazardous substances, such as $H_2S$, in order to improve their reliability and effectiveness.

- All facilities that handle oil and gas with potentially harmful levels of $H_2S$ should have proper medical treatment supplies and trained personnel available and should ensure that first responders, hospitals, and clinics in the area are prepared to treat $H_2S$ exposure.

**Research and Further Studies**

- Further study on the acute exposure levels of $H_2S$ that result in irreversible health effects or lethality in humans should be continued in order to improve emergency planning tools such as atmospheric dispersion models.

- Further research on the effects of surface roughness and obstacles on dense-gas dispersion behavior should be continued to determine their influences on toxic substance concentrations in a dispersing vapor cloud. The Liquefied Gaseous Fuels Spill Test Facility could be used for spill tests to assist in this research.

- EPA should continue to study the issues surrounding worst-case releases, their consequences, and the likelihood of worst-case or other significant releases for extremely hazardous substances and the role and relationship of these issues to prevention, preparedness, and response.

# GLOSSARY

**Abandon:** To cease producing oil or gas from a well when it becomes unprofitable. A wildcat may be abandoned after it has been proven nonproductive. Usually, before a well is abandoned, some of the casing is removed and salvaged and one or more cement plugs are placed in the borehole to prevent migration of fluids between the various formations. In many States, wells may not be abandoned unless approved by an official regulatory agency.

**Accidental Release:** The unanticipated emissions of a regulated substance or other extremely hazardous substance into the air from a stationary source.

**Acid:** Any chemical compound, one element of which is hydrogen, that dissociates in solution to produce free-hydrogen ions. For example, hydrochloric acid, HCl, dissociates in water to produce hydrogen ions, $H^+$, and chloride ions, $Cl^-$.

**Additive:** A substance or compound added in small amounts to a larger volume of another substance to change some characteristic of the latter. In the oil industry, additives are used in lubricating oil, fuel, drilling mud, and cement for cementing casing.

**Air drilling:** A method of rotary drilling that uses compressed air as its circulation medium. This method of removing cuttings from the wellbore is as efficient or more efficient than the traditional methods using water or drilling mud; in addition, the rate of penetration is increased considerably when air drilling is used. However, a principal problem in air drilling is the penetration of formations containing water, since the entry of water into the system reduces its efficiency.

**Alkalinity:** The combining power of a base, or alkali, as measured by the number of equivalents of an acid with which it reacts to form a salt.

**Annular injection:** Long-term disposal of wastes between the outer wall of the drill stem or tubing and the inner wall of the casing or open hole.

**Annulus or annular space:** The space around a pipe in a wellbore, the outer wall of which may be the wall of either the borehole or the casing.

**API:** The American Petroleum Institute. Founded in 1920, this national oil trade organization is the leading standardizing organization on oil-field drilling and production equipment. It maintains departments of transportation, refining, and marketing in Washington, D.C., and a department of production in Dallas.

**Artificial lift:** Any method used to raise oil to the surface through a well after reservoir pressure has declined to the point at which the well no longer produces by means of natural energy. Artificial lift may also be used during primary recovery if the initial reservoir pressure is inadequate to bring the hydrocarbons to the surface. Sucker-rod pumps, hydraulic pumps, submersible pumps, and gas lift are the most common methods of artificial lift.

**Barrel (bbl):** A measure of volume for petroleum products. One barrel (1 bbl) is equivalent to 42 U.S. gallons or 158.97 liters. One cubic meter (1 $m^3$) equals 6.2897 bbl.

**Basin:** A synclinal structure in the subsurface, formerly the bed of an ancient sea. Because it is composed of sedimentary rock and its contours provide traps for petroleum, a basin is a good prospect for exploration. For example, the Permian Basin in West Texas is a major oil producing area.

**Bit:** The cutting or boring element used in drilling oil and gas wells. Most bits used in rotary drilling are roller-cone bits. The bit consists of the cutting element and the circulating element. The circulating element permits the passage of drilling fluid and uses the hydraulic force of the fluid stream to improve drilling rates. In rotary drilling, several drill collars are joined to the bottom end of the drill-pipe column for added weight. The bit is attached to the end of the drill collar.

**Blowdown:** The emptying or depressurizing of a material from a vessel. The material thus discarded.

**Blowout preventer (BOP):** Equipment installed at the wellhead, at surface level on land rigs and on the seafloor of floating offshore rigs, to prevent the escape of pressure either in the annular space between the casing and drill pipe or in an open hole during drilling and completion operations.

**Blow out:** To suddenly expel oil-well fluids from the borehole with great velocity. To expel a portion of water and steam from a boiler to limit its concentration of minerals.

**Borehole:** The wellbore; the hole made by drilling or boring.

**Casing:** Steel pipe placed in an oil or gas well as drilling progresses to prevent the wall of the well from caving in during drilling and to provide a means of extracting petroleum if the well is productive.

**Casing string:** Casing is manufactured in lengths of about 30 ft, each length or joint being joined to another as casing is run in a well. The entire length of all the joints of casing is called the casing string.

**Cement:** A powder consisting of alumina, silica, lime, and other substances which hardens when mixed with water. Extensively used in the oil industry to bond casing to the walls of the wellbore.

**Cement plug:** A portion of cement placed at some point in the wellbore to seal it.

**Christmas tree:** Assembly of fittings and valves at the tip of the casing of an oil well that controls the flow of oil from the well.

**Close-in:** A well capable of producing oil or gas, but temporarily not producing.

**Collar:** A coupling device used to join two lengths of pipe. A combination collar has left-hand threads in one end and right-hand threads in the other. A drill collar.

**Commercial production:** Oil and gas output of sufficient quantity to justify keeping a well in production.

**Completion fluid:** A special drilling mud used when a well is being completed. It is selected not only for its ability to control formation pressure, but also for its properties that minimize formation damage.

**Completion operations:** Work performed in an oil or gas well after the well has been drilled to the point at which the production string of casing is to be set. This work includes setting the casing, perforating, artificial stimulation, production testing, and equipping the well for production. It is done prior to the commencement of the actual production of oil or gas in paying quantities, or in the case of an injection or service well, prior to when the well is plugged and abandoned.

**Corrosion:** A complex chemical or electrochemical process by which metal is destroyed through reaction with its environment. Rust is an example of corrosion.

**Crude oil:** Unrefined liquid petroleum. It ranges in gravity from 9° to 55° API and in color from yellow to black, and it may have a paraffin, asphalt, or mixed base. If a crude oil, or crude, contains a sizable amount of sulfur or sulfur compounds, it is called a sour crude; if it has little or no sulfur, it is called a sweet crude. In addition, crude oils may be referred to as heavy or light according to API gravity, the lighter oils having the higher gravities.

**Cuttings:** The fragments of rock dislodged by the bit and brought to the surface in the drilling mud. Washed and dried samples of the cuttings are analyzed by geologists to obtain information about the formations drilled.

**Demulsify:** To resolve an emulsion, especially of water and oil, into its components.

**Desander:** A centrifugal device used to remove fine particles of sand from drilling fluid to prevent abrasion of the pumps. A desander usually operates on the principle of a fast-moving stream of fluid being put into a whirling motion inside a cone-shaped vessel.

**Desilter:** A centrifugal device, similar to a desander, used to remove very fine particles, or silt, from drilling fluid to keep the amount of solids in the fluid to the lowest possible level. The lower the solids content of the mud, the faster the rate of penetration.

**Disposal well:** A well into which salt water is pumped; usually part of a saltwater-disposal system.

**Drill:** To bore a hole in the earth, usually to find and remove subsurface formation fluids such as oil and gas.

**Drill collar:** A heavy, thick-walled tube, usually steel, used between the drill pipe and the bit in the drill stem to weight the bit in order to improve its performance.

**Drill cutting:** The formation rock fragments that are created by the drill bit during the drilling process.

**Drilling fluid:** The circulating fluid (mud) used in the rotary drilling of wells to clean and condition the hole and to counterbalance formation pressure. A water-based drilling fluid is the conventional drilling mud in which water is the continuous phase and the suspended medium for solids, whether or not oil is present. An oil-based drilling fluid has diesel, crude, or some other oil as its continuous

phase with water as the dispersed phase. Drilling fluids are circulated down the drill pipe and back up the hole between the drill pipe and the walls of the hole, usually to a surface pit. Drilling fluids are used to lubricate the drill bit, to lift cuttings, to seal off porous zones, and to prevent blowouts. There are two basic drilling media: muds (liquid) and gases. Each medium comprises a number of general types. The type of drilling fluid may be further broken down into numerous specific formulations.

**Drill pipe:** The heavy seamless tubing used to rotate the bit and circulate the drilling fluid. Joints of pipe 30 ft long are coupled together by means of tool joints.

**Drill site:** The location of a drilling rig.

**Drill stem:** The entire length of tubular pipes, composed of the kelly, the drill pipe, and drill collars, that make up the drilling assembly from the surface to the bottom of the hole.

**Drill string:** The column, or string, of drill pipe, not including the drill collars or kelly. Often, however, the term is loosely applied to include both the drill pipe and drill collars.

**Emulsion:** A mixture in which one liquid, termed the dispersed phase, is uniformly distributed (usually as minute globules) in another liquid, called the continuous phase or dispersion medium. In an oil-water emulsion, the oil is the dispersed phase and the water the dispersion medium; in a water-oil emulsion the reverse holds. A typical product of oil wells, water-oil emulsion also is used as a drilling fluid.

**Embrittlement:** Through chemical reactions with $H_2S$, steel and other materials become more brittle and more likely to break.

**Emulsion breaker:** A system, device, or process used for breaking down an emulsion and rendering it into two or more easily separated compounds (like water and oil). Emulsion breakers may be (1) devices to heat the emulsion, thus achieving separation by lowering the viscosity of the emulsion and allowing the water to settle out; (2) chemical compounds, which destroy or weaken the film around each globule of water, thus uniting all the drops; (3) mechanical devices such as settling tanks and wash tanks; or (4) electrostatic treaters, which use an electric field to cause coalescence of the water globules. This is also called electric dehydration.

**Enhanced oil recovery (EOR):** A method or methods applied to depleted reservoirs to make them productive once again. After an oil well has reached depletion, a certain amount of oil remains in the reservoir, which enhanced recovery is targeted to produce. EOR can encompass secondary and tertiary production.

**EPA:** United States Environmental Protection Agency.

**Exploration:** The search for reservoirs of oil and gas, including aerial and geophysical surveys, geological studies, core testing, and the drilling of wildcats.

**Extraction:** The physical removal of oil and gas from a well.

**Field:** A geographical area in which a number of oil or gas wells produce from a continuous reservoir. A field may refer to surface area only or to underground productive formations as well. In a single field, there may be several separate reservoirs at varying depths.

**Flare:** Combustion of wastegases, such as $H_2S$ or natural gas, which are not able to be profitably brought to market.

**Flowing well:** A well that produces oil or gas without any means of artificial lift.

**Formation:** A bed or deposit composed throughout of substantially the same kinds of rock; a lithologic unit. Each different formation is given a name, frequently as a result of the study of the formation outcrop at the surface and sometimes based on fossils found in the formation.

**Gas plant:** A plant for the processing of natural gas, by other than solely mechanical means, for the extraction of natural gas liquids, and/or the fractionation of the liquids into natural gas liquid products such as ethane, butane, propane, and natural gasoline.

**Heater-treater:** A vessel that heats an emulsion and removes water and gas from the oil to raise it to a quality acceptable for pipeline transmission. A heater-treater is a combination of a heater, free-water knockout, and oil and gas separator.

**Hydrocarbons:** Organic compounds of hydrogen and carbon, whose densities, boiling points, and freezing points increase as their molecular weights increase. Although composed of only two elements, hydrocarbons exist in a variety of compounds because of the strong affinity of the carbon atom for other atoms and for itself. The smallest molecules of hydrocarbons are gaseous; the largest are solid.

**Ignitability (RCRA):** The hazardous characteristic of ignitability for purposes of RCRA is defined in 40 CFR 261.21 and is generally a liquid with a flash point less than 140 degrees F., a non-liquid that causes fire under a friction condition, an ignitable compressed gas, or is an oxidizer.

**Inhibitor:** An additive used to retard undesirable chemical action in a product. It is added in small quantities to gasolines to prevent oxidation and gum formation, to lubricating oils to stop color change, and to corrosive environments to decrease corrosive action.

**Injection well:** A well in which fluids have been injected into an underground stratum to increase reservoir pressure.

**Kelly:** A pipe attached to the top of a drill string and turned during drilling. It transmits twisting torque from the rotary machinery to the drill string and ultimately to the bit.

**$LC_{50}$ (median lethal concentration):** The concentration of a chemical required to cause death in 50% of the exposed population when exposed for a specified time period, and observed for a specified period of time after exposure. Refers to inhalation exposure concentration in the context of air toxics (may refer to water concentration for tests of aquatic organisms or systems).

**Lease:** A legal document executed between a landowner (or a lessor) and a company or individual, as lessee, that grants the right to exploit the premises for minerals or other products. The area where production wells, stock tanks, separators, and production equipment are located.

**Lowest-observed-adverse-effect level (LOAEL):** The lowest dose or exposure level of a chemical in a study at which there is a statistically or biologically significant increase in the frequency or severity of an adverse effect in the exposed population as compared with an appropriate, unexposed control group.

**Mud:** The liquid circulated through the wellbore during rotary drilling and workover operations. In addition to its function of bringing cuttings to the surface, drilling mud cools and lubricates the bit and drill stem, protects against blowouts by holding back subsurface pressures, and deposits a mud cake on the wall of the borehole to prevent loss of fluids to the formation. Although it originally was a suspension of earth solids (especially clays) in water, the mud used in modern drilling operations is a more complex, three-phase mixture of liquids, reactive solids, and inert solids. The liquid phase may be fresh water, diesel oil, or crude oil and may contain one or more conditioners.

**Natural gas:** Naturally occurring mixture of hydrocarbon and non-hydrocarbon gases found in geologic formations beneath the earth's surface. The principal hydrocarbon constituent is methane.

**No-observed-adverse-effect level (NOAEL).** The highest experimental dose at which there is no statistically or biologically significant increases in frequency or severity of adverse health effects, as seen in the exposed population compared with an appropriate, unexposed population. Effects may be produced at this level, but they are not considered to be adverse.

**Odor perception threshold:** The lowest concentration at which a substance is first able to be smelled.

**Oil base muds:** A drilling fluid that is a water-oil emulsion with oil as the continuous phase. The oil content ranges from 50-98% oil. Oil muds are used to reduce drilling torque and to stabilize reactive shales that impede the drilling process.

**Oil and gas separator:** An item of production equipment used to separate the liquid components of the well stream from the gaseous elements. Separators are vertical or horizontal and are cylindrical or spherical in shape. Separation is accomplished principally by gravity, the heavier liquids falling to the bottom and the gas rising to the top. A float valve or other liquid-level control regulates the level of oil in the bottom of the separator.

**Oil field:** The surface area overlying an oil reservoir or reservoirs. Commonly, the term includes not only the surface area but also the reservoir, wells, and production equipment.

**Operator:** The person or company, either proprietor or lessee, actually operating an oil well or lease.

**Packer:** A piece of downhole equipment, consisting of a sealing device, a holding or setting device, and an inside passage for fluids. It is used to block the flow of fluids through the annular space between the tubing and the wall of the wellbore by sealing off the space. The packer is usually made up in the tubing string some distance above the producing zone. A sealing element expands to

prevent fluid flow except through the inside bore of the packer and into the tubing. Packers are classified according to configuration, use, and method of setting and whether or not they are retrievable (i.e., whether they can be removed when necessary, or whether they must be milled or drilled out and thus destroyed).

**Perforate:** To pierce the casing wall and cement to provide holes through which formation fluids may enter, or to provide holes in the casing so that materials may be introduced into the annulus between the casing and the wall of the borehole. Perforating is accomplished by lowering into the well a perforating gun, or perforator, that fires electrically detonated bullets or shaped charges from the surface.

**Permeability:** A measure of the ease with which fluids can flow through a porous rock.

**pH:** A measure of the acidity or alkalinity of a solution, numerically equal to 7 for neutral solutions, increasing with increasing alkalinity and decreasing with increasing acidity.

**Primary recovery:** Oil production in which only existing natural energy sources in the reservoir provide for movement of the well fluids to the wellbore.

**Produced water:** The water (brine) brought up from the hydrocarbon-bearing strata during the extraction of oil and gas. It can include formation water, injection water, and any chemicals added downhole or during the oil/water separation process.

**Producing zone:** The zone or formation from which oil or gas is produced.

**Production:** The phase of the petroleum industry that deals with bringing the well fluids to the surface and separating them. Production also includes storing, gauging, and otherwise preparing the product for the pipeline.

**Production casing:** The last string of casing or liner that is set in a well, inside of which is usually suspended the tubing string.

**RCRA (Resource Conservation and Recovery Act):** The Federal statute enacted in 1976 (and subsequent amendments) which amended the Solid Waste Disposal Act. Among other things, RCRA and its amendments established and/or augmented three significant programs: the hazardous waste management program, the solid waste program, and the underground storage tank program.

**Reference concentration (RfC):** An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

**Reservoir:** A subsurface, porous, permeable rock body in which oil or gas or both are stored. Most reservoir rocks are limestones, dolomites, sandstones, or a combination of these. The three basic types of hydrocarbon reservoirs are oil, gas, and condensate. An oil reservoir generally contains three fluids--gas, oil, and water--with oil the dominant product. In the typical oil reservoir, these fluids occur in different phases because of the variance in their gravities. Gas, the lightest, occupies the upper part of the reservoir rocks; water occupies, the lower part; and oil occupies, the intermediate section. In addition to occurring as a cap or in solution, gas may accumulate

independently of the oil; if so, the reservoir is called a gas reservoir. Associated with the gas, in most instances, are salt water and some oil. In a condensate reservoir, the hydrocarbons may exist as a gas, but when brought to the surface, some of the heavier ones condense to a liquid or condensate. At the surface the hydrocarbons from a condensate reservoir consist of gas and a high gravity crude (i.e., the condensate). Condensate wells are sometimes called gas-condensate reservoirs).

**Rig:** The derrick, drawworks, and attendant surface equipment of a drilling or workover unit.

**Routine emissions:** The anticipated emissions of a regulated substance or other extremely hazardous substance into the air from a stationary source during its normal operation.

**Secondary recovery:** Any method by which an essentially depleted reservoir is restored to producing status by the injection of liquids or gases (from extraneous sources) into the wellbore. This injection effects a restoration of reservoir energy, which moves the formerly unrecoverable secondary reserves through the reservoir to the wellbore.

**Shale shaker:** A series of trays with sieves that vibrate to remove cuttings from the circulating fluid in rotary drilling operations. The size of the openings in the sieve is carefully selected to match the size of the solids in the drilling fluid and the anticipated size of cuttings. It is also called a shaker.

**Short-term exposure limit (STEL):** A time-weighted average that the American Conference of Government and Industrial Hygienists (ACGIH) indicates should not be exceeded any time during the work day. Exposures at the STEL should not be longer than 15 minutes and should not be repeated more than 4 times per day. There should be at least 60 minutes between successive exposure at the STEL.

**Shut-in well:** A non-producing well with its pump turned off, and the stuffing box closed, which has been inspected to ensure there is no leakage.

**Sour:** Containing hydrogen sulfide or caused by hydrogen sulfide or another sulfur compound.

**Stripper well:** A well nearing depletion that produces a very small amount of oil or gas.

**Tail gas:** gas that leaves a sulfur recovery process after most of the $H_2S$ has been converted to $SO_2$.

**Tank battery:** A group of production tanks located in the field, used for storage of crude oil.

**Tertiary recovery:** A recovery method used to remove additional hydrocarbons after secondary recovery methods have been applied to a reservoir. Sometimes more hydrocarbons can be removed by injecting liquids or gases (usually different from those used in secondary recovery and applied with different techniques) into the reservoir.

**Threshold limit value (TLV):** The concentration of a substance below which no adverse health effects are expected to occur for workers, assuming exposure for 8 hours per day, 40 hours per week. TLVs are published by the American Conference of Governmental Hygienists (ACGIH). This listing may be useful in identifying substances used in the workplace and having the potential to be emitted into the ambient air.

**Time-weighted average (TWA):** An approach to calculating the average exposure over a specified time period.

**Tubing:** Small-diameter pipe that is run into a well to serve as a conduit for the passage of oil and gas to the surface.

**Uncertainty factor (UF):** One of several, generally 10-fold factors, applied to a NOAEL or a LOAEL to derive a reference dose (RfD) from experimental data. UFs are intended to account for (a) the variation in the sensitivity among the members of the human population; (b) the uncertainty in extrapolating animal data to humans; (c) the uncertainty in extrapolating from data obtained in a less-than-lifetime exposure study to chronic exposure; and (d) the uncertainty in using a LOAEL rather than a NOAEL for estimating the threshold region.

**Volatile:** Readily vaporized.

**Waterflood:** A method of secondary recovery in which water is injected into a reservoir to remove additional quantities of oil that have been left behind after primary recovery. Usually, a waterflood involves the injection of water through wells specially set up for water injection and the removal of the water and oil from the wells drilled adjacent to the injection wells.

**Wellbore:** A borehole; the hole drilled by the bit. A wellbore may have casing in it or may be open (i.e., uncased); or a portion of it may be cased and a portion of it may be open.

**Well completion:** The activities and methods necessary to prepare a well for the production of oil and gas; the method by which a flow line for hydrocarbons is established between the reservoir and the surface. The method of well completion used by the operator depends on the individual characteristics of the producing formation or formations. These techniques include open-hole completions, conventional perforated completions, sand-exclusion completions, tubingless completions, multiple completions, and miniaturized completions.

**Wellhead:** The equipment used to maintain surface control of a well, including the casinghead, tubing head, and Christmas tree.

**Workover:** One of more of a variety of remedial operations performed on a producing oil well to try to increase production. Some examples of workover operations are deepening, plugging back, pulling and resetting the liner, and squeeze-cementing.

**Workover fluids:** A special drilling mud used to keep a well under control when it is being worked over. A workover fluid is compounded carefully so it will not cause formation damage.

# APPENDIX A

# BACKGROUND INFORMATION ON THE OIL AND GAS PRODUCTION INDUSTRY

# APPENDIX A

# BACKGROUND INFORMATION ON THE OIL AND GAS PRODUCTION INDUSTRY

## EXPLORATION AND DEVELOPMENT

Although geological and geophysical studies provide information about potential accumulations of petroleum, only exploratory drilling can confirm the presence of petroleum.

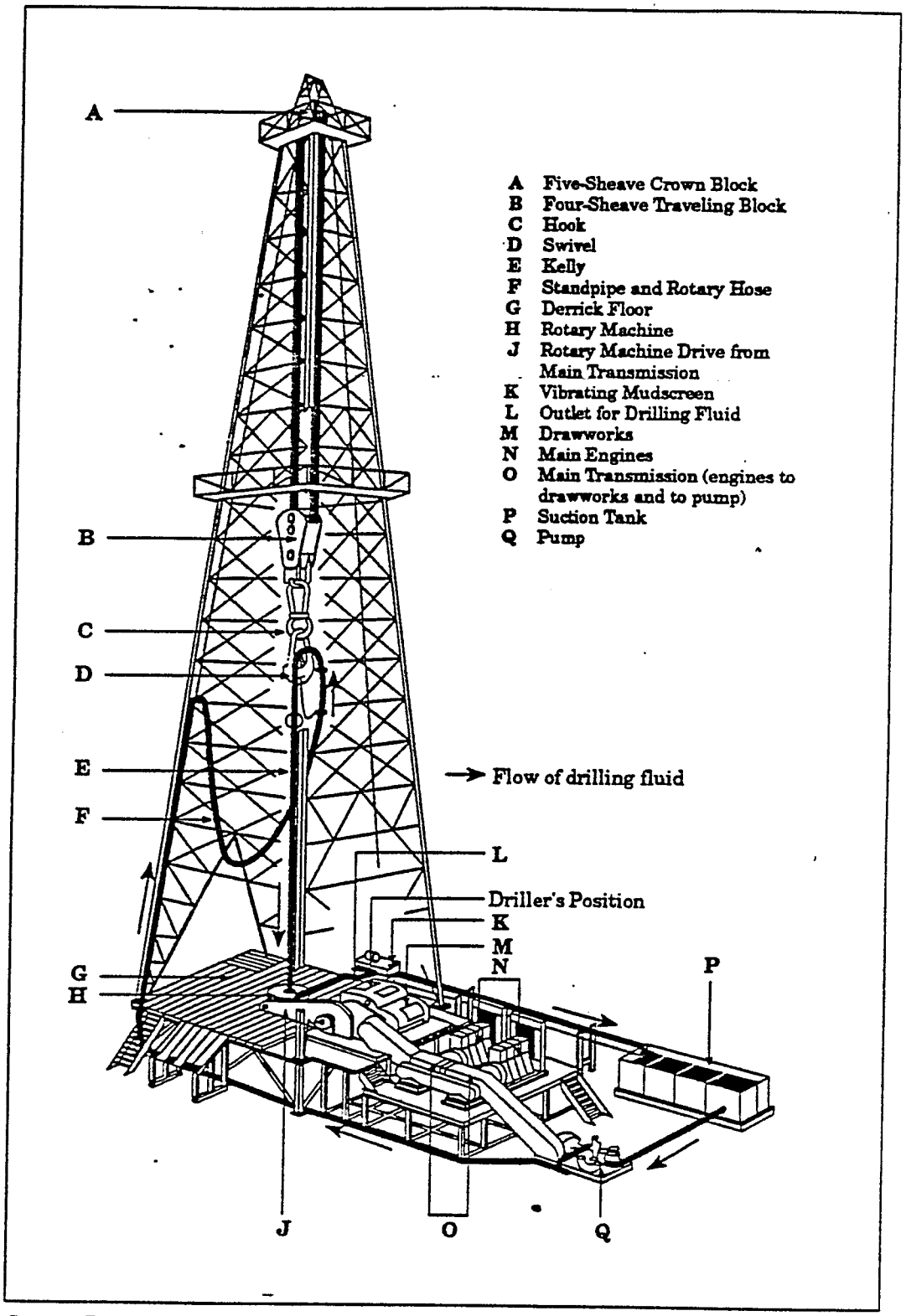
Rotary drilling, the primary drilling method in the United States, provides a safe way to control high-pressure oil/gas/water flows and allow for the simultaneous drilling of the well and removal of cuttings. This makes it possible to drill wells over 30,000 feet deep. Figure A-1 illustrates the process. Most rotary drilling operations employ a circulation system using a water- or oil-based fluid, called "mud" because of its appearance. The mud is pumped down the hollow drill pipe and across the face of the bit to provide lubrication and remove cuttings. Cuttings are removed at the surface by shale shakers, desanders, and desilters; they are then deposited in the reserve pit excavated or constructed next to the rig. Air drilling, which is considerably faster and less expensive than drilling with water- or oil-based fluids, is used in areas where high pressure or water-bearing formations are not anticipated.

Potential producing zones are normally measured and analyzed during exploratory drilling. If evidence of hydrocarbons is found, a drill stem test can show whether commercial quantities of oil and gas are present. If so, the well is prepared for production. This is called "completion." The most common method is the "cased hole" completion. Production casing is run into the hole and cemented permanently in place. Then one or more strings of production tubing are set in the hole, productive intervals are isolated with packers, and surface equipment is installed. The well is not actually completed until a gun or explosive charge perforates the production casing and begins the flow of petroleum into the well (U.S. EPA, 1987). Figure A-2 shows a cross section of a common well.

While a well is being drilled, heavy fittings have to be installed at the surface where the casing is attached, as each string of casing is inserted into the hole. Each part of the casing head is supported by a part of the casing head which was installed at the top of the next larger string of casing when it was run (U.S. EPA, 1987).

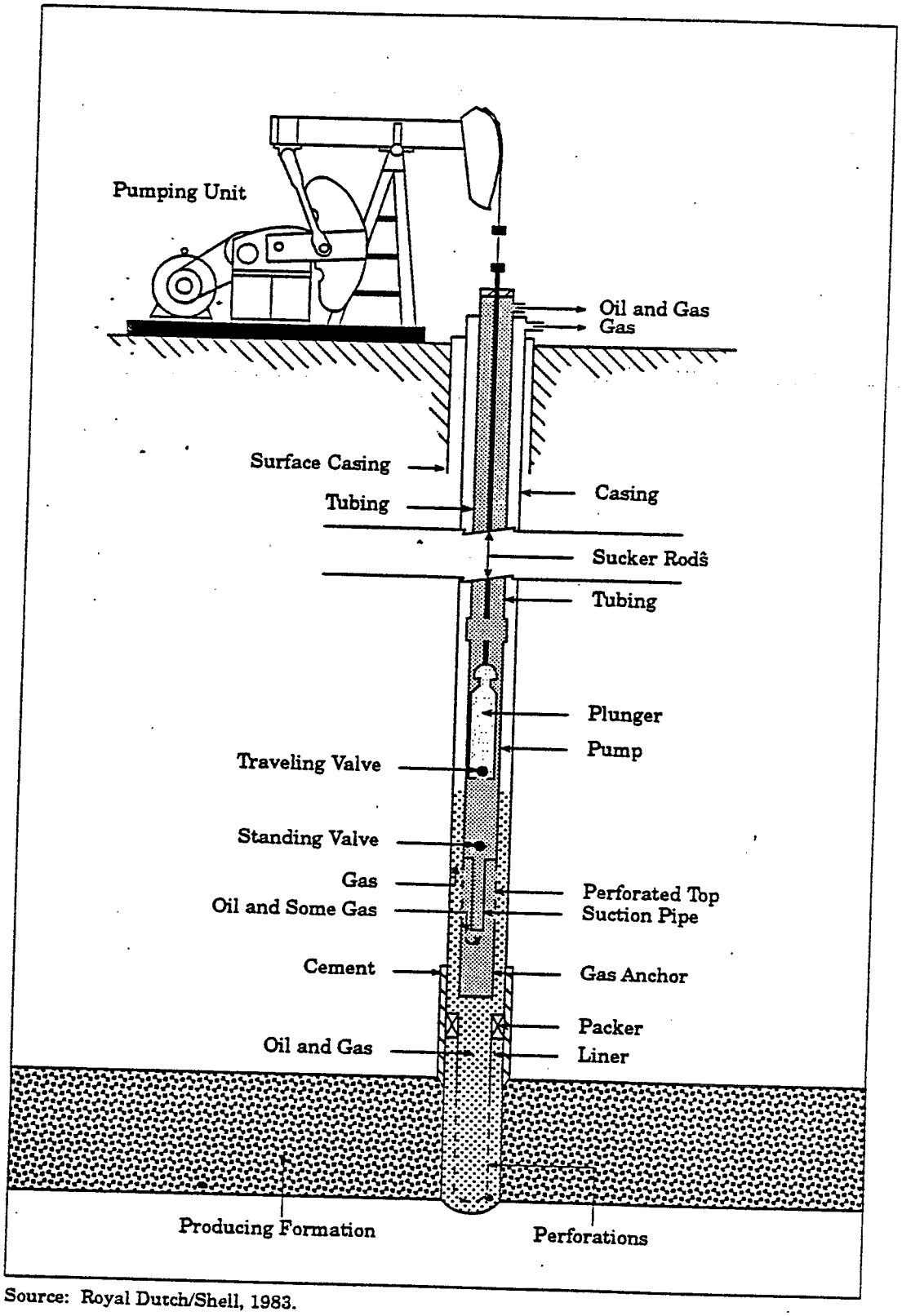
## HOW OIL AND GAS ARE PRODUCED

Production operations generally include all activities associated with the recovery of oil and gas from geologic formations. They can be divided into activities associated with downhole operations and activities associated with surface operations. Downhole operations include primary secondary, and tertiary recovery methods, well workovers, and well stimulation activities. Activities associated with surface operations include oil/gas/water




Source: Royal Dutch/Shell, 1983.

**Figure A-1. Rotary drilling rig.**




Source: Royal Dutch/Shell, 1983.

**Figure A-2. Cross section of a well pumping installation.**

separation, fluid treatment, and disposal of produced water. The term "extraction" is commonly used to refer to activities associated with getting oil or gas to the surface; production includes both extraction and the surface operations involved in processing the materials extracted from the well. Production, as discussed in this report, is limited to the processing and storage that occurs at the well site. Transportation and further processing is not included in the scope of this report.

**Downhole Operations**

The initial production of oil or gas from the reservoir is called primary recovery. Natural pressure or artificial lift methods (surface or subsurface pumps and gas lifts) are used to bring the gas or oil out of the formation and to the surface (see Figure A-3). High-pressure gas can also be injected to lift the oil from the reservoir.

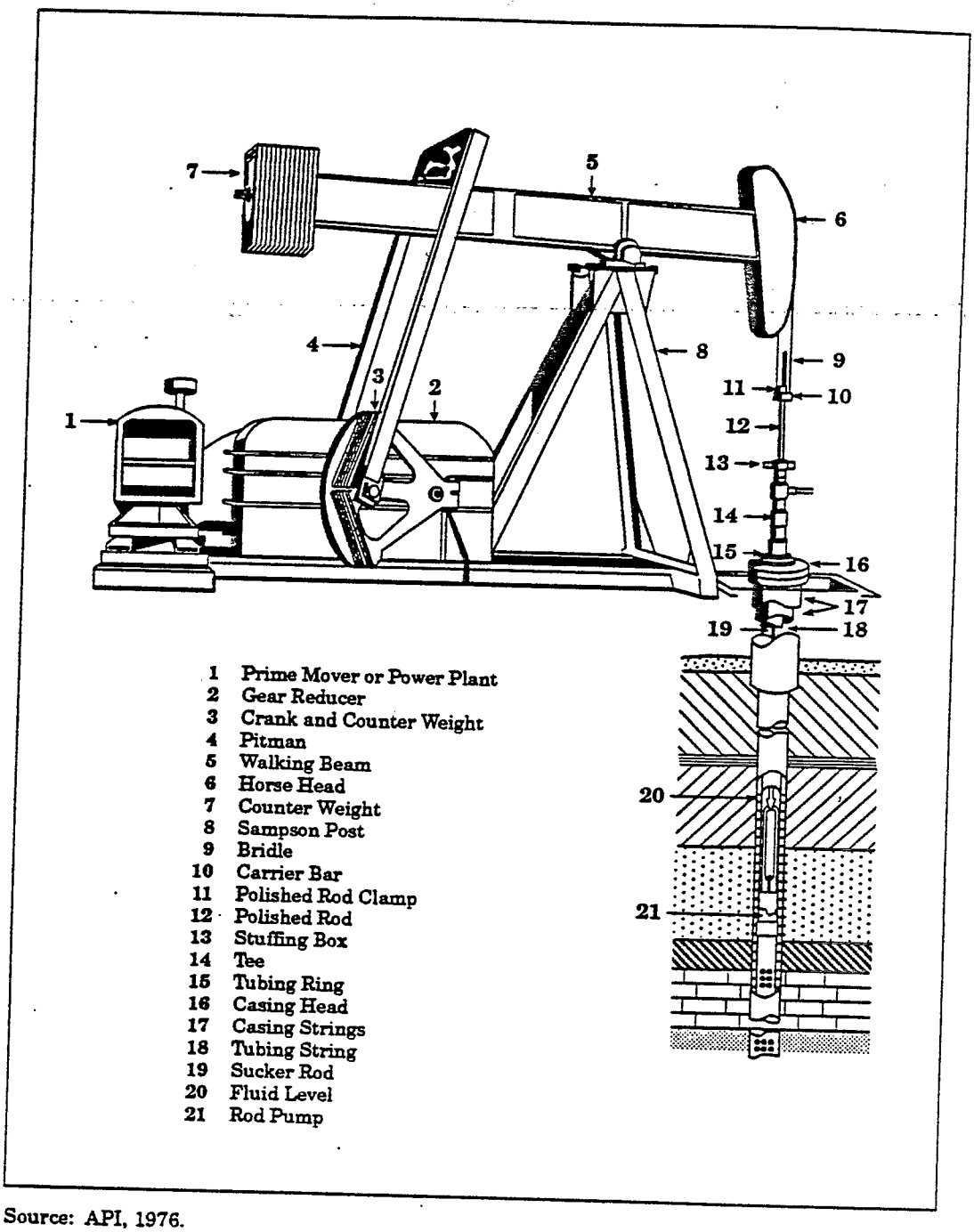
During the primary recovery stage, natural pressure in the reservoir may decline and artificial lift may be needed. One of three general types of pumps may be used: (1) pumps at the bottom of the hole run by a string of rods; (2) pumps at the bottom of the hole run by high-pressure liquids; and (3) bottom-hole centrifugal pumps (API, 1976).

The pumping unit includes a complete set of surface equipment that imparts an up-and-down motion to the sucker-rod string, which is connected the bottom-hole pump. Figure A-2 shows the parts of such as unit. Deep wells often require the long-stroke pumping provided by hydraulic units.

A stuffing box is used in a pumping well to pack or seal off the pressure inside the tubing so that liquid and gas cannot leak outside the polished rod. A stuffing box consists of flexible material or packing housed in a box which provides a method of compressing the packing. The packing material gradually wears out and must be replaced before it loses its effectiveness as a seal (API, 1976).

Primary recovery methods alone can produce oil and gas from most reservoirs, but over the life of the well production gradually decreases. Some form of secondary recovery will eventually be needed in nearly all wells. Secondary recovery methods inject gas or liquid into the reservoir to maintain pressure. The most frequent method is waterflooding, which involves injecting treated water (seawater, fresh water or produced water) into the formation through a separate well.

When secondary recovery methods are no longer adequate, the last portion of the oil that can be economically produced is recovered by tertiary methods. These include chemical, physical, and thermal methods or some combination. Chemical methods involve injection of fluids containing substances such as surfactants and polymers. Miscible oil recovery methods inject gases such as carbon dioxide and natural gas that combine with the oil. Thermal recovery methods include steam injection and *in situ* combustion (or "fire flooding"). The injected gases or fluids from secondary and tertiary recovery operations are




Source: API, 1976.

**Figure A-3. Main parts of a pumping unit.**

dissolved or mixed with the oil produced by the well and must be removed during surface production operations (U.S. EPA, 1987).

Workovers are another type of downhole production operation. Workovers are used to restore or increase production when downhole mechanical failures or blockages, such as sand or paraffin deposits have inhibited the flow of a well. Fluids circulated into the well for a workover must be compatible with the formation and must not adversely affect permeability. The workover fluid may be reclaimed or disposed of when the well is put back into production. Workover fluids are similar to completion fluids, which are special fluids used when the well is completed (ready for the production phase), to minimize formation damage and control potential problems such as $H_2S$ corrosion.

Other chemicals are used periodically or continuously to inhibit corrosion, reduce friction, or simply keep the well flowing (U.S. EPA, 1987).

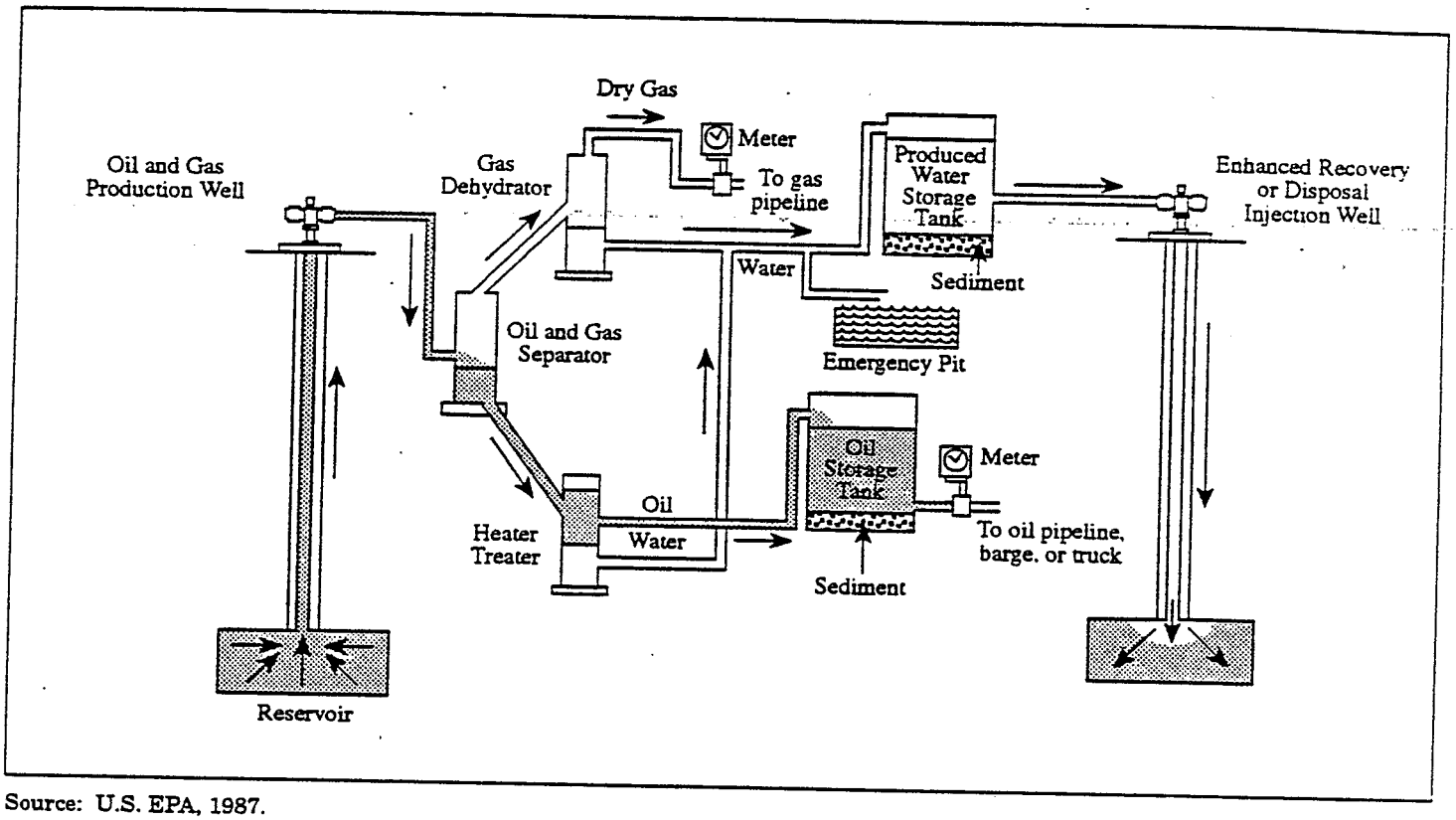
**Surface Operations**

As fluids are pumped to the surface, they are collected and treated to separate the various components (oil, gas, gas liquids, and water). Figure A-4 shows the separation process. These surface operations become more complex as secondary and tertiary recovery methods are employed. The ratio of water and other fluids to oil tends to increase as producing reservoirs are depleted. In new wells little or no water may be produced. The volume of water produced by stripper wells varies greatly. Stripper wells may produce more than 100 barrels of water for every barrel of oil, especially if waterflooding is used as a secondary recovery (U.S. EPA, 1987).

Separation involves the use of equipment to separate the gas, oil and water from each other. The actual separation may be accomplished in a single step or several steps depending on the relative amounts and the physical characteristics of the material which is delivered to the surface. Complete separation may require several stages involving different pressures, temperatures, and possibly additives if the material is delivered to the surface at a high pressure and the oil and gas are present in an emulsion.

After separation, the gas is transported by pipeline to a gas processing facility if the quantities from a specific well are adequate. If the quantities are inadequate, the gas is flared (burned). Gas processing facilities remove inerts ($N_2$, $CO_2$), hydrogen sulfide ($H_2S$), and liquids (oil and water) to produce pipeline quality gas which has a nominal heating value of 1000 BTU per cubic foot. Gas can also be re-injected into the well if necessary to help manage the reservoir or the production from the well.

Oil that is recovered from the separators at the well is placed in tanks and transported to a refinery for processing. This transportation is by pipeline if the quantities are adequate to justify installation of a pipeline or by truck if the production is small.




Source: U.S. EPA, 1987.

**Figure A-4. Typical extraction operation showing separation of oil, gas, and water.**

Water recovered from the separators at the well is placed in tanks or pools. This water will ultimately be reinjected into the producing formation, injected into a disposal well, or discharged. Reinjection into the producing formation and injection into a disposal well are the most common methods for water disposal; discharge is rarely used. Permits are usually required for these water disposition options.

The equipment used at the surface to control the well is called the well head. If high production or significant gas pressure is expected, the well head is usually built of cast or forged steel, and machined to a close fit. These sealed fittings prevent well fluids from blowing or leaking at the surface. Parts of the well head may be designed to hold pressures up to 20,000 lb per sq in (psi). Some well heads are just simple assemblies to support the weight of the tubing in the well, and may not be built to hold pressure. For stripper wells, or other low-production, low-pressure wells, a simple well head can be used as long as only small amounts of gas are produced with the oil (API, 1976).

High pressures or corrosive gases such as $H_2S$ require well heads with special valves and control equipment to control the flow of oil and gas from the well. These are constructed of heavy metal and installed above the casing head or tubing head before the well is completed. This collection of valves is called a Christmas tree because of its shape and the large number of fittings branching out above the well head. The tree diverts fluids through alternative chokes (API, 1976).

Safety measures should be adequate to prevent high pressure wells from going out of control. Equipment is available that automatically shuts off production if there is damage to the wellhead or to automatic surface safety valves at the wellhead.

Simpler types of Christmas trees can be used on low pressure or pumping wells. Pressure gauges on the well head and Christmas tree measure the pressure in the casing and tubing. If the pressures under various operating conditions are known, better control can be maintained (API, 1976).

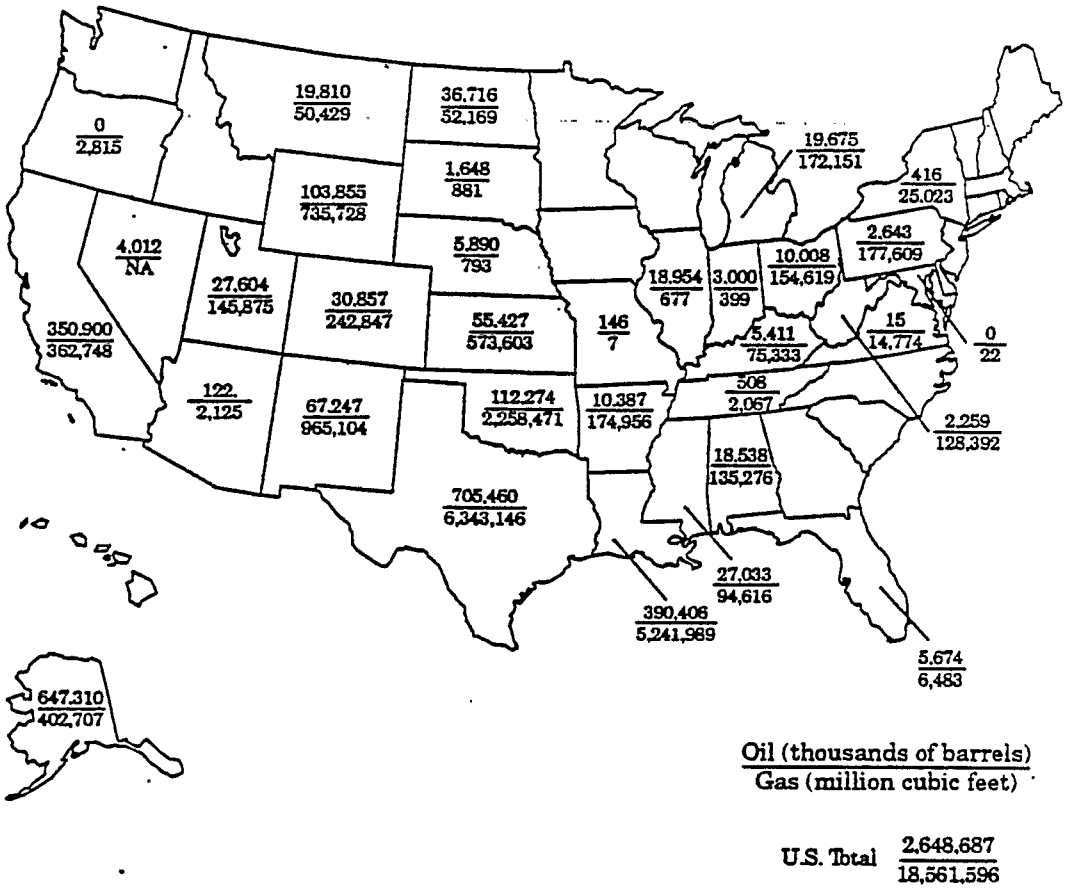
## OVERVIEW OF THE INDUSTRY

The U.S. petroleum industry drilled its first oil well in 1859. Since that first well, the oil and gas industry has grown to be extremely complex and diverse. In 1990, approximately 869,887 wells in over 33 States were producing oil and gas in the United States. The oil and gas obtained from these wells is found at depths ranging from 30 feet to 30,000 feet below the earth's surface. The major U.S. areas of onshore production include the southwest (including California), the midwest, and Alaska, with lesser contributions from the Appalachians. Table A-1 lists production estimates for the oil and gas producing States. In 1990-1991, Texas led all States in oil and natural gas production, turning out 705 million barrels of oil and 6.3 trillion cubic feet of natural gas (Petroleum Independent, 1992). Figure A-5 shows U.S. oil and gas production by State. The bar graph in Figure A-6 shows distribution of States containing more than 70 percent of gas wells in the U.S. Some of these

**Table A-1. 1991 Oil and 1990 Gas Production Estimates**

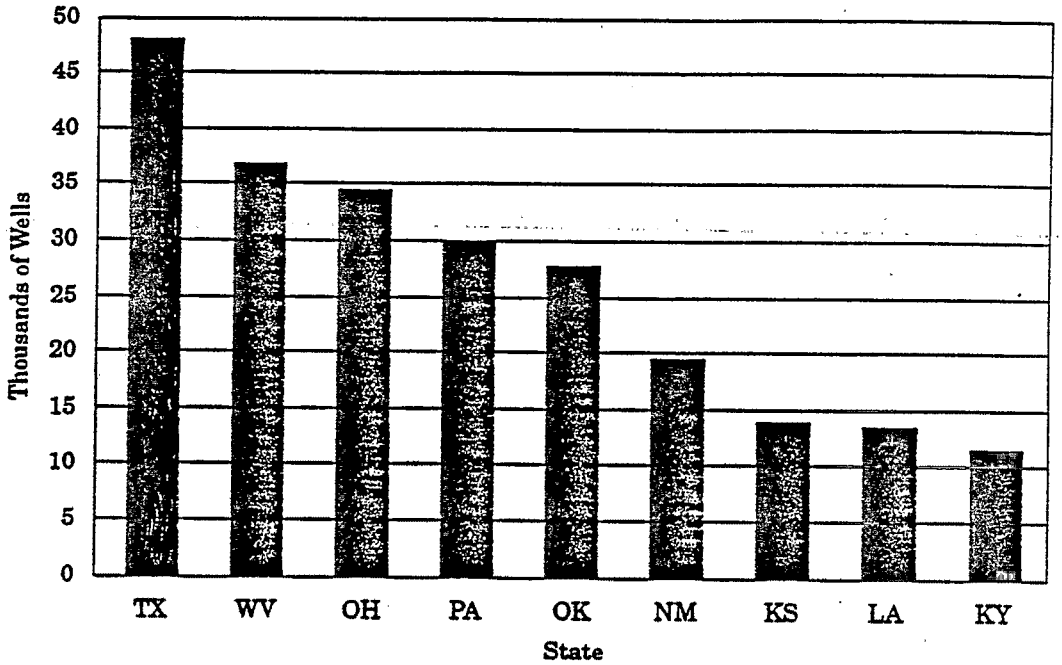
| State | Number of Producing Oil Wells[a] | Oil Production (thousands of barrels)[b] | Number of Producing Gas Wells[a] | Gas Production (million cubic feet)[b] |
|---|---|---|---|---|
| Alabama | 872 | 18,538 | 2,038 | 135,276 |
| Alaska | 1,466 | 647,310 | 109 | 402,907 |
| Arizona | 22 | 122 | NA | 2,125 |
| Arkansas | 7,265 | 10,387 | 3,460 | 174,956 |
| California | 43,375 | 350,900 | 1,169 | 362,748 |
| Colorado | 6,596 | 30,857 | 5,097 | 242,897 |
| Florida | 83 | 5,674 | NA | 6,483 |
| Illinois | 31,874 | 19,954 | 356 | 677 |
| Indiana | 7,506 | 3,000 | 1,311 | 399 |
| Kansas | 45,470 | 55,427 | 14,043 | 573,603 |
| Kentucky | 22,741 | 5,411 | 11,713 | 75,333 |
| Louisiana | 23,812 | 390,406 | 13,530 | 5,241,989 |
| Maryland | 0 | 0 | NA | 22 |
| Michigan | 4,570 | 19,675 | 1,438 | 172,151 |
| Mississippi | 2,168 | 27,033 | 629 | 94,616 |
| Missouri | 854 | 146 | NA | 7 |
| Montana | 3,854 | 19,810 | 2,428 | 50,429 |
| Nebraska | 1,440 | 5,890 | NA | 793 |
| Nevada | 46 | 4,012 | NA | NA |
| New Mexico | 18,546 | 67,247 | 19,537 | 965,104 |
| New York | 4,043 | 416 | 5,406 | 25,023 |
| North Dakota | 3,546 | 36,716 | 103 | 52,169 |
| Ohio | 30,089 | 10,008 | 34,697 | 154,619 |
| Oklahoma | 95,468 | 112,274 | 27,919 | 2,258,471 |
| Oregon | 0 | 0 | NA | 2,815 |
| Pennsylvania | 22,338 | 2,643 | 30,000 | 177,609 |
| South Dakota | 149 | 1,648 | 52 | 881 |
| Tennessee | 736 | 508 | 527 | 2,067 |
| Texas | 188,829 | 705,460 | 48,075 | 6,343,146 |
| Utah | 1,972 | 27,604 | 742 | 145,875 |
| Virginia | 25 | 15 | 819 | 14,774 |
| West Virginia | 15,950 | 2,143 | 37,000 | 178,000 |
| Wyoming | 11,397 | 103,855 | 2,431 | 735,728 |
| Federal Waters | 4,468 | NA | 3,591 | NA |
| Other | 25 | NA | 147 | NA |
| U.S. | 601,520 | 2,684,687 | 268,367 | 18,561,596 |

Combined Source: Petroleum Independent, September 1992, attributes the individual column sources to:
[a] World Oil.
[b] Energy Information Administration.
–NA Not available.



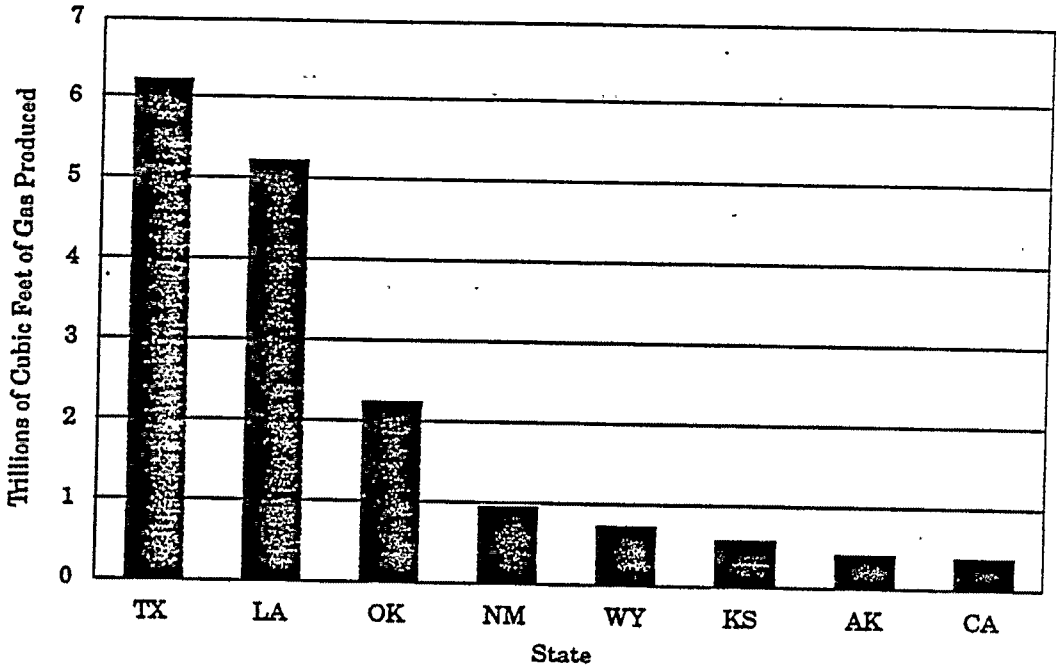

Combined Source: Petroleum Independent, September 1992, attributes the oil and gas sources to: World Oil Energy Information Administration.

**Figure A-5. 1991 U.S. oil and gas production by State.**




Source: World Oil (in Petroleum Independent, September 1992)

**Figure A-6. States with the most producing gas wells in 1990.**




Source: Energy Information Administration (in Petroleum Independent, September 1992).

**Figure A-7. Gas production in 1990 from the top producing states.**

States, however, are not the largest gas producers. Figure A-7 shows that Texas, Louisiana, Oklahoma, New Mexico, Wyoming, Kansas, Alaska, and California account for 92 percent of domestic gas production. Alaska, California, Louisiana, and Texas account for 78 percent of domestic oil production.

**Principal Production Industry Groups**

The industry can be divided into four groups. The first group consists of the major oil companies. These companies are highly vertically integrated, which means that they perform both "upstream" activities (oil exploration, development and production) and "downstream" activities (transportation, refining and marketing).

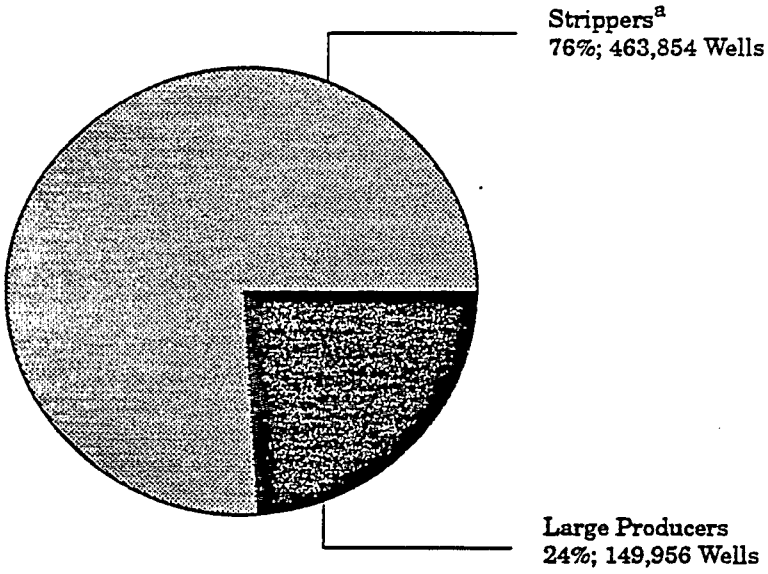
The second group is the large independents. These companies primarily explore, develop, and produce oil and gas, but do not perform downstream activities. Some large independents produce oil and gas only, while others provide such additional services as contract drilling and pipeline operations.

The third group is the small independents. Little information is available that would characterize this group quantitatively. However, small independents are known to have fewer wells and/or lower production wells. The lower operating expenses of small independents makes it more affordable to continue producing small quantities from low volume wells.

The fourth group consists of companies that provide a variety of specialized services to the oil and gas drilling rigs and platforms, such as designing, manufacturing, and installing specialized hardware. They also provide geophysical support, drilling mud, and logging services.

**Diversity of Production**

Production from individual wells varies greatly from a high of 11,500 barrels per day to less than 10 barrels per day. As shown in Figure A-8, over 70 percent of U.S. oil wells are "stripper" wells. The definition of a stripper well varies from State to State. However, these wells are generally defined as wells that produce 10 barrels of oil per day or less, or 100 thousand cubic feet (mcf) of gas per day or less. In 1990, 463,854 stripper wells existed and produced a total of 383,197,000 barrels of oil (NSWA, 1991). Stripper well production is shown in Table A-2. Figure A-9 shows that stripper wells produced 14 percent of the 2,684,687,000 barrels of oil produced in the United States in 1990 (U.S. EIA, 1991; U.S. EIA, 1987). Figure A-10 shows the proportion of stripper wells in the 10 States with the largest numbers of wells overall. In all 10 States, stripper wells comprised more 50 percent of producing wells. However, Figure A-11 demonstrates that in the 10 top oil producing States, oil from stripper wells is relatively low in volume. These wells typically are near depletion of recoverable natural resources and produce only a small quantity of oil or gas.




[a] Strippers are defined as those producing 10 barrels a day or less.

Source: Interstate Oil and Gas Compact Commission and National Stripper Well Association.

**Figure A-8. Number of producing oil wells in the U.S. in 1990.**

**Table A-2. 1990 Oil Production from Stripper Wells by State**

| Location | Number of Producing Wells[a] | Number of Producing Stripper[c] Wells | Percentage of Producing Wells Which Are Stripper Wells* | Amount of Crude Oil Produced (thousands of barrels)[b] |
|---|---|---|---|---|
| Alabama | 872 | 514 | 58% | 18,538 |
| Alaska | 1,466 | 0 | 0% | 647,310 |
| Arizona | 22 | 12 | 55% | 122 |
| Arkansas | 7,265 | 7,290 | NA* | 10,387 |
| California | 43,375 | 26,128 | 60% | 350,900 |
| Colorado | 6,596 | 5,234 | 79% | 30,857 |
| Florida | 83 | 0 | 0% | 5,674 |
| Illinois | 31,874 | 33,700 | NA* | 19,954 |
| Indiana | 7,506 | 5,764 | 77% | 3,000 |
| Kansas | 45,470 | 45,227 | 99% | 55,427 |
| Kentucky | 22,741 | 19,330 | 85% | 5,411 |
| Louisiana | 23,812 | 17,695 | 74% | 390,406 |
| Michigan | 4,570 | 3,967 | 87% | 19,675 |
| Mississippi | 2,168 | 615 | 28% | 27,033 |
| Missouri | 854 | 375 | 44% | 146 |
| Montana | 3,854 | 3084 | 80% | 19,810 |
| Nebraska | 1,440 | 1,269 | 88% | 5,890 |
| Nevada | 46 | 0 | 0% | 4,012 |
| New Mexico | 18,546 | 15,261 | 82% | 67,247 |
| New York | 4,043 | 3,748 | 93% | 416 |
| North Dakota | 3,546 | 1,205 | 34% | 36,716 |
| Ohio | 30,089 | 29,576 | 98% | 10,008 |
| Oklahoma | 95,468 | 73,345 | 77% | 112,274 |
| Pennsylvania | 22,338 | 21,800 | 98% | 2,643 |
| South Dakota | 149 | 26 | 17% | 1,648 |
| Tennessee | 736 | 923 | NA* | 508 |
| Texas | 188,829 | 127,790 | 68% | 705,460 |
| Utah | 1,972 | 1,026 | 52% | 27,604 |
| Virginia | 25 | 22 | 88% | 15 |
| West Virginia | 15,950 | 15,975 | NA | 2,143 |
| Wyoming | 11,397 | 2,953 | 26% | 103,855 |
| U.S. | 601,520 | 463,854 | 77% | 2,684,687 |

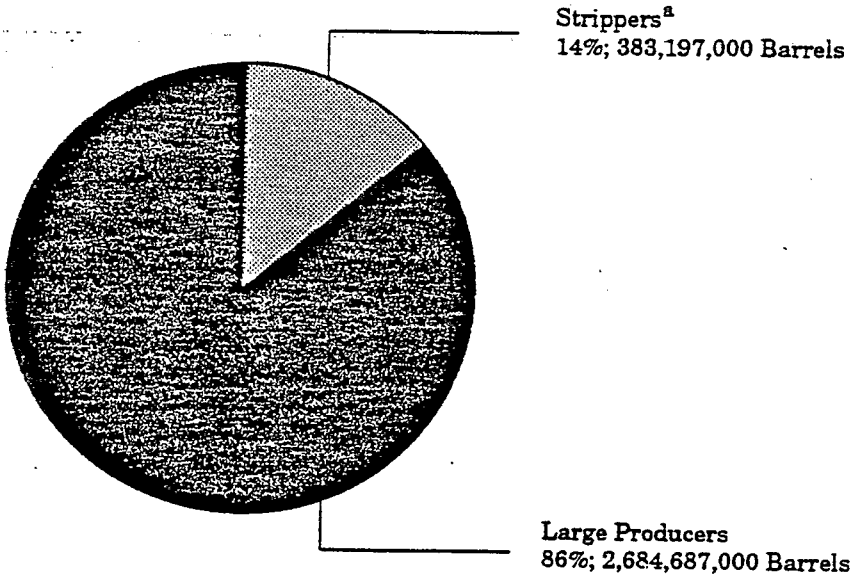
Combined Source: Petroleum Independent, September 1992, attributes the individual column sources to:
[a] World Oil.
[b] Energy Information Administration.
[c] Interstate Oil and Gas Compact Commission and National Stripper Well Association.

*Petroleum Independent warns "[number of producing stripper wells] data cannot be compared to "Producing Oil Wells" table due to different sources and technology."

NA Unable to calculate.

Table A-2. 1990 Oil Production from Stripper Wells by State (continued)

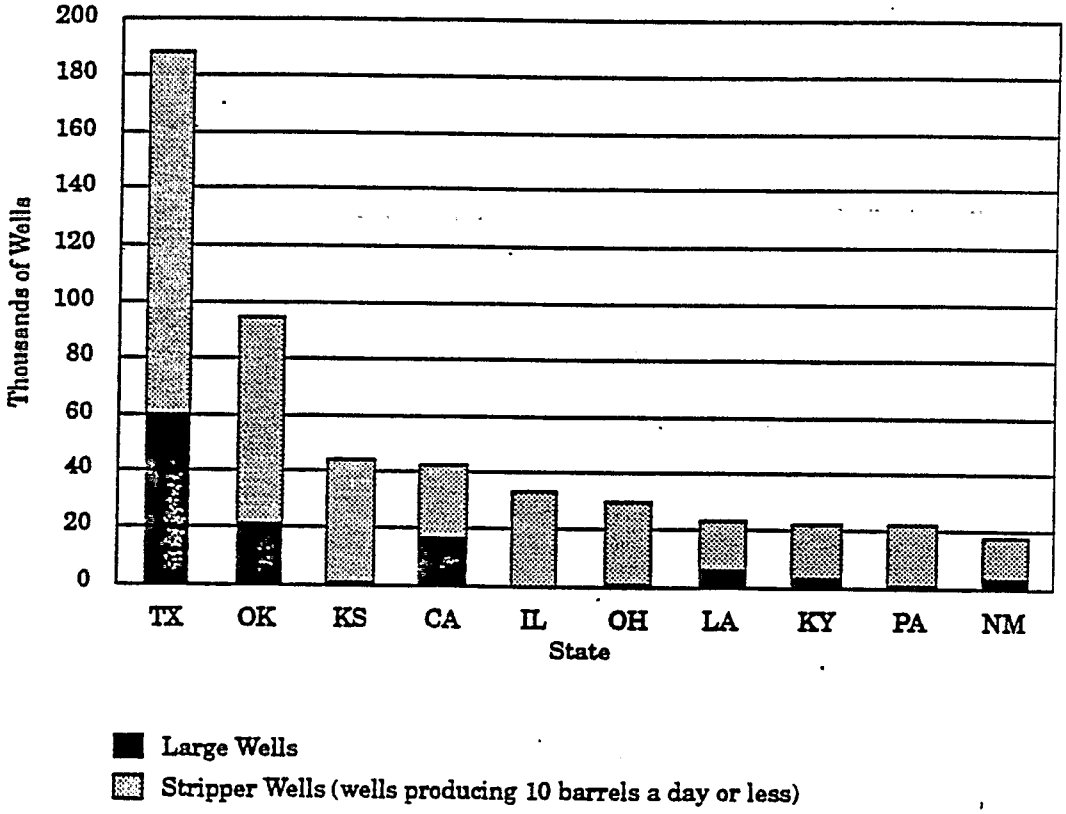
| Amount of Crude Oil Produced from Stripper Wells (thousands of barrels)[c] | Percentage of Crude Oil Produced from Stripper Wells |
|---|---|
| 1,486 | 8% |
| 0 | 0% |
| 26 | 21% |
| 5,693 | 55% |
| 36,405 | 10% |
| 5,698 | 19% |
| 0 | 0% |
| 18,520 | 93% |
| 3,002 | NA* |
| 40,873 | 74% |
| 4,338 | 80% |
| 7,154 | 2% |
| 4,599 | 23% |
| 802 | 3% |
| 120 | 82% |
| 2,449 | 12% |
| 2,011 | 34% |
| 0 | 0% |
| 14,296 | 21% |
| 383 | 92% |
| 2,053 | 6% |
| 7,271 | 73% |
| 78,599 | 70% |
| 2,622 | 99% |
| 64 | 4% |
| 419 | 83% |
| 135,850 | 19% |
| 1,035 | 4% |
| 12 | 80% |
| 2,122 | 99% |
| 5,297 | 5% |
| 389,197 | 14% |




[a] Strippers are defined as those producing 10 barrels a day or less.

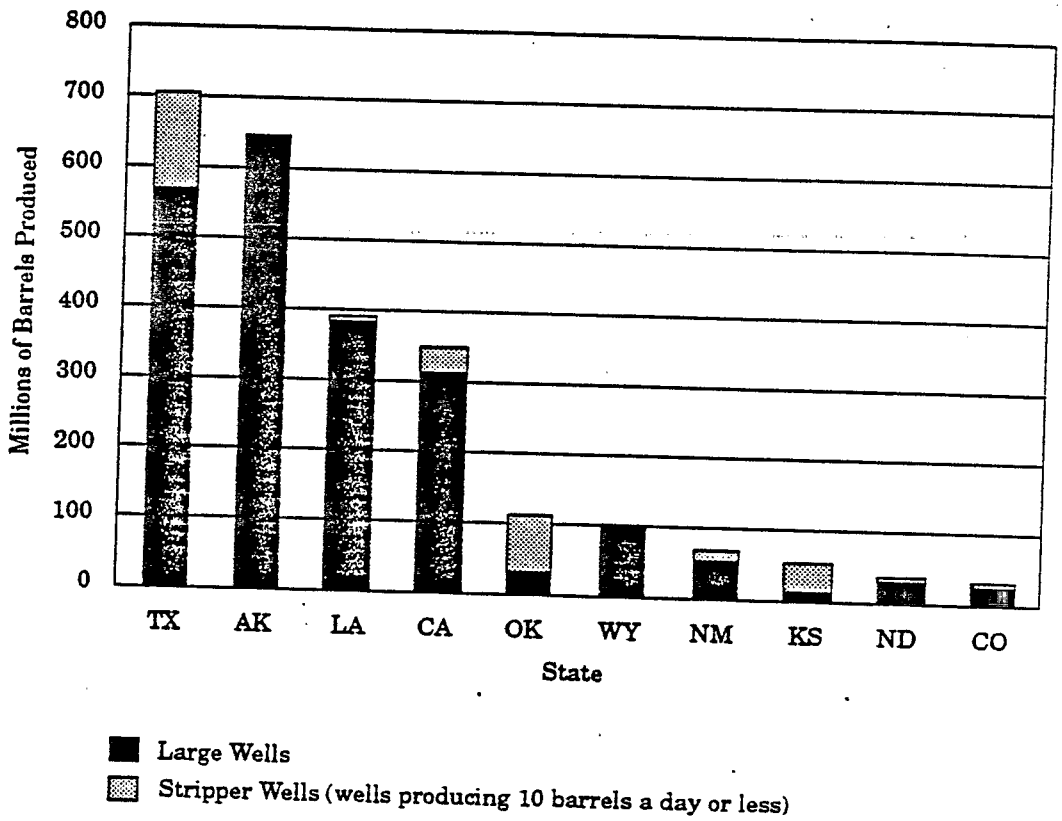
Source: Interstate Oil and Gas Compact Commission and National Stripper Well Association.

**Figure A-9. 1990 U.S. oil production.**




Source: World Oil (in Petroleum Independent, September 1992).

**Figure A-10. States with the largest number of producing oil wells in 1990.**




Source: World Oil (in Petroleum Independent, September 1992).

**Figure A-11. Oil production in 1990 from the top producing states.**

## REFERENCES


API. 1976. *Primer on Oil and Gas Production.* American Petroleum Institute, Committee on Vocational Training and Executive Committee on Training and Development. American Petroleum Institute, Dallas, TX.

API. 1983. *Introduction to Oil and Gas Production.* 4th ed. American Petroleum Institute, Committee on Vocational Training and Executive Committee on Training and Development. American Petroleum Institute, Dallas, TX.

IOGCC. 1990. *The Interstate Oil and Gas Compact Committee Bulletin,* Volume IV, Number 2. The Oil and Gas Compact Commission, Dallas, TX.

NSWA. 1991. *National Stripper Well Survey.* National Stripper Well Association.

Petroleum Independent. 1992. The Oil and Natural Gas Producing Industry in Your State, 1992-1993. *Petroleum Independent*, Vol. 62. No. 7. Independent Petroleum Association of America.

Royal Dutch/Shell Group of Companies. 1983. *The Petroleum Handbook*, 6th ed. Elsevier Science Publishers B.V., Amsterdam, The Netherlands, 38, 52.

U.S. EIA. 1990. *Natural Gas Annual.* U.S. Energy Information Administration.

U.S. EIA. 1991. *Petroleum Supply Annual.* U.S. Energy Information Administration.

U.S. EPA. 1987. *Management of Wastes from the Exploration, Development, and Production of Crude Oil, Natural Gas, and Geothermal Energy, Volume I*, EPA/530-SW-88 003. U.S. Environmental Protection Agency, Research Triangle Park, NC.

*World Oil Magazine*, Forecast Review Issue. February 1992.

# APPENDIX B

## SUBJECTS OF STATE $H_2S$ REGULATIONS AND GUIDELINES

**Table B-1. Subjects of State $H_2S$ Regulations and Guidelines**

| Regulations and Guidelines | Oklahoma | Texas | Michigan | California* |
|---|---|---|---|---|
| Characteristics and Effects of $H_2S$ (including emergency rescue, resuscitators, effects on metal and artificial respiration) | NA | ● | ● | ● |
| Initial Testing | ● | NA | ● | ● |
| Periodic Gas Analyses | ● | NA | NA | NA |
| Nuisance Odors | ● | NA | NJ | NA |
| Guidelines for Safe Drilling Operations | | | | |
| A. Location Requirements | ● | ● | ● | ● |
| B. Drilling Equipment (Including blowout preventer, controls, piping and accessories, etc.) | ● | ● | ● | ● |
| C. Monitoring Equipment (including alarm systems and gas detection equipment) | ● | ● | ● | ● |
| D. Personal Protective Equipment (including all personnel, breathing apparatus, equipment specs., etc.) | ● | ● | ● | ● |
| E. Employee Physical Requirements | NA | ● | NA | NA |
| F. Training Requirements | ● | ● | ● | NA |
| G. Drills and Orientations | ● | ● | ● | ● |
| H. Maintenance of Equipment | ● | ● | ● | ● |
| I. Warning Systems | ● | ● | ● | ● |
| J. Evacuation | ● | ● | ● | ● |
| Guidelines for Safe Production Operations | | | | |
| A. Applicability | NA | ● | ● | NA |
| B. General Provisions | NA | ● | ● | NA |
| 1. Concentration Determination | ● | ● | ● | NA |
| 2. Radius of Evacuation (ROE) | ● | ● | ● | ● |
| 3. Escape Rate Volume Determinators | ● | ● | ● | NA |
| 4. Storage Tank Provisions | ● | ● | ● | NA |
| 5. . . . ppm ROE in excess of . . . feet | ● | ● | ● | ● |
| 6. Implementation | ● | ● | ● | NA |
| 7. Control and Safety Equipment | ● | ● | ● | ● |
| 8. Contingency Plan | ● | ● | ● | ● |
| 9. Training | ● | ● | ● | NA |
| 10. Injection Provision | ● | ● | ● | NA |
| 11. Certificate of Compliance Provision | ● | ● | ● | NA |
| 12. Accident Notification | ● | ● | ● | ● |

NA Not available in reviewed literature.
• The subject was identified under the State's $H_2S$ regulations or guidelines.
A Rule 36 references API RP19.
NJ Not under Congressional jurisdiction.
MMIOSH Required by Michigan OSHA.
*CA grants the supervisor of the Oil and Gas Division discretionary authority to control $H_2S$ releases.

# APPENDIX C

# ATMOSPHERIC DISPERSION CALCULATIONS FOR $H_2S$ RELEASES FROM OIL AND GAS EXTRACTION FACILITIES

# APPENDIX C

# ATMOSPHERIC DISPERSION CALCULATIONS FOR $H_2S$ RELEASES FROM OIL AND GAS EXTRACTION FACILITIES

## INTRODUCTION

The purpose of this appendix is to provide supporting details for the analyses of atmospheric dispersion of $H_2S$ conducted for this report. In Chapter III, computer models were used, together with information on published studies of sour gas releases, to examine the range of predictions of the distances of concern for scenarios of $H_2S$ releases from wellheads or pipelines. The inputs to the analyses are reviewed, and the outputs of three sample calculations for two of the scenarios are described. Outputs for a horizontal wellhead release are described for calculations using the SLAB and SAPLUME models. The output for a vertical wellhead release using the DEGADIS model is also described.

## SUMMARY INPUT AND OUTPUT DATA

Summary data for the wellhead blowout and pipe rupture scenarios are presented in Tables C-1 and C-2, respectively. As described in Chapter III, analyses for wellhead blowouts were conducted using the SLAB, DEGADIS, and SAPLUME models. Analyses of the pipe rupture scenarios were conducted using the SACRUNCH and SAPLUME models.

The wellhead blowout scenarios in Chapter III result from various assumed flow rates as presented again in Table C-1. The following discussion presents some justification for the choice of these flow rates. Flow rates are functions of such factors as rock permeabilities, gas properties, depth, and tube and casing diameters. In practice, there are large variabilities in these parameters. One measure of the potential rate of flow from a well is the Calculated Absolute Open Flow Rate (CAOF), which is the rate of flow of gas into the well bore when the pressure is atmospheric. A sample of 15 wells in western Wyoming had CAOFs with a geometrical mean of $4.7 \times 10^6$ standard cubic feet per day (scf/d) or $1.3 \times 10^5$ cubic meters per day ($m^3/d$) (Layton et al., 1983). The 95 percent confidence interval spanned the range from $2.1 \times 10^5$ scf/d ($5.9 \times 10^3$ $m^3/d$) to $10^8$ scf/d ($2.8 \times 10^6$ $m^3/d$). Alp et al. (1990) considered CAOFs of between $5 \times 10^4$ and $5 \times 10^6$ $m^3/d$ as representative of wells in Alberta and chose $10^6$ $m^3/d$ as representative for the purposes of risk analysis. The Quest report (1992) considered CAOFs in the range $2.2 \times 10^5$ to $7.3 \times 10^6$ $m^3/d$ for a system of wells in southwestern Wyoming. The actual flow rates out of a ruptured well will be less than the CAOF because of frictional effects in the pipework. By contrast, the Quest report and Layton et al. use the CAOF as a conservative estimate of flow rate. Based on the above discussions, a flow rate of $2 \times 10^7$ scf/d was chosen for representative calculations, with a flow rate of $10^8$ scf/d being taken as an example of a very high flow rate.

## TABLE C-1

## SUMMARY OF INPUT AND OUTPUT DATA
## WELLHEAD BLOWOUT SCENARIOS

| SCENARIO[a] | A | B | C | D | D(E)* |
|---|---|---|---|---|---|
| INPUTS | | | | | |
| Flow rate ($m^3/d$) | $6x10^5$ | $6x10^5$ | $6x10^5$ | $6x10^5$ | $3x10^6$ |
| Vol. % $H_2S$ | 7.5 | 27 | 15 | 30 | 30 |
| Density[b] @ 0°C ($kg/m^3$) | 0.862 | 1.293 | 1.038 | 1.128 | 1.128 |
| Release temperature (°C) | 0 | 0 | 0 | 0 | 0 |
| Total release rate (kg/s) | 5.99 | 8.98 | 7.21 | 7.83 | 39.2 |
| Release rate of $H_2S$ (kg/s) | 0.79 | 2.85 | 1.58 | 3.17 | 15.8 |
| Ambient temperature (°C) | 5 | 5 | 5 | 5 | 5 |
| Relative humidity (%) | 75 | 75 | 75 | 75 | 75 |
| Atmospheric stability category | F | F | F | F | F |
| Windspeed (m/s) | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Surface roughness length (m) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Effective area of release ($m_2$) | 0.02 | 0.02 | 0.02 | 0.02 | 0.1 |
| OUTPUTS: HORIZONTAL RELEASE | | | | | |
| SLAB: | | | | | |
| Distance to: | | | | | |
| $LC_{01}$(m) | 700 | 2,800 | 1,500 | 2,900 | 7,000 |
| ERPG-2(m) | 2,800 | 7,000 | 4,700 | 7,000 | >10,000 |
| SAPLUME: | | | | | |
| Distance to: | | | | | |
| $LC_{01}$ (m) | 1,000 | 2,700 | 1,500 | 3,000 | >10,000 |
| ERPG-2(m) | 3,100 | 10,000 | 5,700 | 10,000 | >10,000 |
| OUTPUTS: VERTICAL RELEASE | | | | | |
| SLAB: | | | | | |
| Distance to: | | | | | |
| $LC_{01}$(m) | 0 | 0 | 0 | 0 | 0 |
| ERPG-2(m) | 0 | 0 | 0 | 0 | 0 |
| DEGADIS: | | | | | |
| Distance to: | | | | | |
| $LC_{01}$(m) | 0 | 0 | 0 | 0 | 0 |
| ERPG-2(m) | 0 | 0 | 0 | 0 | 0 |
| SAPLUME: | | | | | |
| Distance to: | | | | | |
| $LC_{01}$(m) | 0 | 0 | 0 | 0 | 0 |
| ERPG-2(m) | 0 | 0 | 0 | 0 | 0 |

[a] Scenarios from Table III-7.
[b] For comparison, density of air @ 0°C=1.293 $kg/m^3$.
* E=Extreme Case.

## TABLE C-2

## PIPE RUPTURE SCENARIOS
## INPUTS AND OUTPUTS (SADENZ MODEL)

| Parameters/Scenario | Composition A[a], rupture of 4" diameter pipeline[b] | Composition D[a], rupture of 16" diameter pipeline[c] |
|---|---|---|
| INPUTS | | |
| Total mass released (kg)[d] | 640 | 31,000 |
| Total mass of $H_2S$ (kg) | 84 | 12,500 |
| Duration of release (s)[d] | 16 | 310 |
| Density @ 0°C (kg/m³) | 0.862 | 1.128 |
| Release temperature (°C) | 0 (32 °F) | 0 (32 °F) |
| Ambient temperature (°C) | 5 (41 °F) | 5 (41 °F) |
| Relative humidity (%) | 75 | 75 |
| Atmospheric stability category | F | F |
| Windspeed (m/s) | 1.5 | 1.5 |
| Surface roughness length(m) | 0.1 | 0.1 |
| OUTPUTS | | |
| Distance to: | | |
| $LC_{01}$ (m) | 600 | 4,300 |
| ERPG-2 (m) | 750 | 5,600 |

[a] Composition from Table III-5.
[b] Spacing between emergency shutdown valves is 1,000 m.
[c] Spacing between emergency shutdown valves is 3,000 m.
[d] From Figure III-22.

Table C-1 also presents values for the effective area of release. These values are derived by dividing the volumetric release rate by the velocity of release and were not the bases for the release scenarios. As stated in Chapter III, the velocity of release was assumed to be "choked," or limited, to sonic velocity (approximately 330 m/s) as a result of the high initial gas pressure.

The temperature of the gas in a well prior to expansion to atmospheric pressure through a rupture depends on the depth of the gas reservoir. The amount of cooling that results from expansion to atmospheric pressure as a result of release depends on the initial pressure and the composition. Alp et al. (1990) assume a representative release temperature of 15°C (288 K) at atmospheric pressure. In the Quest report, the authors assume a reservoir temperature of 60°C and calculate expansion temperatures of between -9°C and 3°C. The calculated results of wellhead blowout and pipeline rupture scenarios in this study are based on a representative release temperature of 0°C. This temperature is below the assumed ambient temperature of 5°C.

Atmospheric conditions characterized by low turbulence and low wind speed provide for decreased dilution of a released chemical with the surrounding air. Thus, these conditions are directionally conservative in terms of potential exposure to accidental releases. Atmospheric thermal stability, impacted by the difference between surface and air temperatures, is often described by Pasquill atmospheric stability categories. These categories range from high turbulence (A) through low turbulence (F). The "F" category is typical of still, nighttime conditions (AIChE, 1989). This category was chosen for the calculations conducted to conservatively evaluate the wellhead blowout and pipeline rupture scenarios. Wind speeds of less than 2 m/s are considered low and create little turbulence. The calculations used in this study's analyses assume a wind speed of 1.5 m/s to conservatively simulate nonturbulent conditions. Actual conditions of A - D stability and higher wind speeds will cause more rapid dilution of an accidental release and will result in a decreased affected distance. The assumption that conditions of low wind speed and stable atmospheric conditions exist uniformly for extended distances also provides conservatism to the analyses.

Terrain is another factor that may influence atmospheric dispersion of a release. The surface roughness length is a measure of the "roughness" of the terrain. Roughness is a function of the type of terrain and the presence of such features as trees and buildings. The models in this study assume that the study of the behavior of dense gas flow around obstacles and through rough terrain is controversial and is an area where further research is needed. Rough terrain will cause more turbulence to atmospheric flows above it than smooth terrain. The value of surface roughness length, 0.1 m, used in the calculated dispersion predictions, is considered to be an intermediate roughness length and typical of highly vegetated rural terrain. It should be noted that lower, more conservative values would be more appropriate in flat, barren terrain.

## SAMPLE SLAB CALCULATIONS

### SLAB Input

The following illustrates how the input is prepared for SLAB, using composition D from Table III-5 as an example. The SLAB input is displayed on Table C-3. The SLAB users' manual provides further guidance (Ermak, 1989).

Line 1: IDSPL is the spill source type. For an evaporating pool, IDSPL =1. For a horizontal jet release IDSPL=2. For a vertical jet release IDSPL=3. For a puff, IDSPL = 4. For the present example, the release is assumed to be horizontal, IDSPL=2.

Line 2: NCALC is a numerical substep parameter. The code developer recommends using NCALC=1. However, NCALC can be increased if numerical stability problems are encountered.

Line 3: WMS is the molecular weight of the wellhead gas in kg/gmole. From Table III-6, it is 0.0252 kg/gmol (from 25.2 g/gmol). Note, however, that the value given in Table C-3 is 0.0289 kg/gmol, for the following reason. Initially, the dilution of the plume is dominated by entrainment caused by its high momentum (its initial velocity equals that of sound). There is considerable dilution in this early phase and, by the time it is over, the density of the plume is only slightly less than that of the surrounding atmosphere. Work on marginally buoyant plumes shows that they are not likely to lift off the ground (Briggs, 1973). However, SLAB runs with WMS = 0.0252 kg/gmol show predicted plume rise that continues to a height of over 100 m. This is regarded as physically unrealistic and the computer model is "fooled" into ignoring plume rise by setting WMS equal to the effective molecular weight of air which is 28.9 g/gmol (0.0 289 kg/gmol). As noted above, this is thought to be physically realistic. The results predicted in this way will be conservative if plume rise does in fact take place.

Line 4: CPS is the vapor heat capacity at constant pressure. Similar to the above molecular weight calculation, the gas mixture vapor heat capacity is calculated by summing the product of the constituents' mole percent and vapor heat capacity. For composition D it is approximately 1,500 J/kg/K.

Line 5: TBP is the boiling point of the released material. For a pure vapor release, SLAB does not in fact use this quantity, which has been arbitrarily set equal to the boiling point of methane, 111.5K.

Line 6: CMEDO is the liquid mass fraction in the initial release and is set to zero because the release is pure vapor.

Lines 7, 8: DHE=509,880 (J/kg) and CPSL=3,349 (J/kg/K) are the heat of vaporization and the liquid specific heat for methane. Their values are taken from Table 2 of the SLAB

Users' Guide. When the released material is pure vapor, as it is in the present case, and the temperature of the cloud does not drop below the boiling point, these values are adequate because the liquid properties will not be used in the SLAB calculation. However, a value for all SLAB input properties must be specified whether they are used or not.

Line 9: RHOSL is the liquid density of the released material. This is another quantity that is not used in the calculations. It has been set equal to the density of water (1,000 kg/m$^3$).

Lines 10,11: SPB and SPC are parameters that go into the saturated vapor pressure formula:

$$P_s = P_a * \exp[SPA - SPB/(T + SPC)],$$

where $P_s$ is the saturated vapor pressure, $P_a$ is the ambient pressure ($1.01 \times 10^5$ N/m$^2$), SPA is defined in the code and T is the local cloud temperature. Table 2 of the SLAB Users' Guide contains some values of SPB and SPC, but not for the mixture modeled here. When these values are unknown, the Users' Guide recommends default values of SPB = -1 and SPC = 0. The code then uses the Clapeyron equation to define the value of SPB. When the released material is pure vapor, as it is in the present case, and the temperature of the cloud does not drop below the boiling point, this default is adequate because the saturation pressure will not be used in the SLAB calculation. However, a value for all SLAB input properties must be specified whether they are used or not.

Lines 12-17: These lines specify the spill parameters. TS is the temperature of the released material, taken to be 273K. QS is the rate of release, estimated at 20 million scfd (7.69 kg/s). AS is the effective area of the release, $1.93 \times 10^{-2}$ m$^2$, obtained by dividing the volumetric flow rate by the speed of sound (340 m/s). TSD is the duration of the release, 3,600 s, the assumed duration of release for a wellhead blowout. QTIS is zero except when modeling an instantaneous puff release. Finally, HS is the height of the release, arbitrarily taken to be 5 m (close to the ground).

Line 18: TAV is the concentration averaging time. This is set equal to 3,600 to be consistent with the exposure time of concern.

Line 19: XFFM is the maximum downwind extent of the calculation. A value of 10 km is used in order to obtain cloud concentration results at large distances away from the release. It is set to $2 \times 10^4$ m, which should be enough to ensure that any results of interest lie within this distance.

Lines 20-23: ZP(I) allows the user to specify up to four heights at which the concentration is calculated as a function of downwind distance. ZP(1) is set to 1.6 m (approximate head elevation above grade). The remaining ZP(I)s are zero, which means that SLAB only considers the first height.

Lines 24-29: These lines specify the meteorological conditions. ZO is the surface roughness length, which is set to 0.1 m, depicting a relatively smooth surface. ZA is the height at

which the windspeed is measured (10 m). UA is the windspeed at height ZA (1.5 m/s). TA is the ambient temperature (273K). RH is the relative humidity (75%, chosen as being typical of Category F weather conditions). Finally, STAB is the stability class (F=stable). The weather conditions (Category F with a low windspeed of 1.5 m/s) have been chosen to simulate unfavorable (close to worst case) conditions.

Line 30: TER is the end of file designator. TER < 0 terminates the run.

## SLAB Output

A partial SLAB output corresponding to the inputs of Table C-3 is given in Table C-4. The interpretation is as follows. The first column gives the downwind distance, x. The second column gives the time at which the maximum concentration arrives at x and the third gives the duration of cloud passage. As can be seen, the duration of passage remains equal to the duration of release until the cloud has traveled several kilometers downwind. The fourth column gives the approximate half-width of the plume, bbc. The remaining six columns give the average concentration (volume fraction) at a height of 1.6 m (as chosen in the SLAB input) for six off-axis distances that are multiples of bbc, 0.5, 1.0, 1.5, etc. The predicted concentrations are zero close in because the plume was arbitrarily released at a height of 5 m. As the plume broadens, the concentrations at height 5 m rise above zero to a maximum at about 25 m to 30 m downwind and then begin to decline as the plume dilutes further.

The effective ERPG-2 is 100 ppm and the effective $LC_{01}$ is about $4.7 \times 10^5$ ppb. These number values are derived as follows: the ERPG-2 for pure $H_2S$ for an exposure time of 1 hour is $3 \times 10^4$ ppb. The volume concentration of $H_2S$ in composition D is 30 percent (see Table III-5). Therefore, the overall concentration of the total released material when the $H_2S$ in it is at $3 \times 10^4$ ppb is $30/0.3 = 1 \times 10^5$ ppb. Similarly, the $LC_{01}$ for pure $H_2S$ is $1.4 \times 10^5$ ppb for an exposure time of 1 hour (see Chapter III). Therefore, the effective $LC_{01}$ for the plume is $140/0.3 = 4.7 \times 10^5$ ppb. As explained in Chapter III, the ERPG-2 is regarded as a threshold at which emergency response might be necessary and the $LC_{01}$ is an approximate threshold for the occurrence of fatalities among the affected population. Reading down the column headed "y/bbc=0," the concentrations first fall below $4.7 \times 10^5$ ppb (= a volume fraction of $4.7 \times 10^{-4}$) at a distance of about 3 km and below $1 \times 10^5$ ppb (= a volume fraction of $1.0 \times 10^{-4}$) at a distance of about 7 km.

## SAMPLE DEGADIS CALCULATIONS

## DEGADIS Input

Table C-5 displays the DEGADIS input for the same case as was prepared for SLAB in Table C-3 except that DEGADIS can only simulate a vertical jet release. The chosen values for most of the parameters have already been explained in the section on SLAB.

Lines 1-4 allow the user to input up to four lines of title.

Line 5 requires the windspeed (1.5 m/s) and the height at which the windspeed is measured (10 m).

Line 6 gives the surface roughness length (0.1 m).

Line 7 requires the parameter INDVEL, the atmospheric stability category (F=6) and the Monin-Obukhov length RML. For INDVEL=1 (the present case) the model calculates RML from the stability category and the surface roughness length, so the user does not need to specify a value for RML.

Line 8 requires the ambient temperature (273K), the ambient pressure (1 atmosphere) and the relative humidity (75%).

Line 9 gives the surface temperature, which is here set equal to the ambient temperature (273K).

Line 10 is a name for the released gas, in this case CPD for ComPosition D.

Line 11 is the molecular weight, 25.2.

Line 12 is the averaging time, taken to be equal to the duration of release, 3,600 s. It is used to calculate the increase in the effective width of the plume as a function of exposure time.

Line 13 is the temperature of the released gas, 273K.

Line 14 contains the upper level of concern (470 ppm, expressed as a volume fraction), the lower level of concern (100 ppm) and the height at which the concentrations are calculated (1.6 m).

Line 15 contains first a variable INDHT=0, meaning that heat transfer from the ground is not included, which does not matter here because the plume, air, and ground all have the same temperature. The second entry is the specific heat of the released gas at constant pressure (1,500 J/kg/K). The third entry, CPP=0, indicates that an approximation was made in which the specific heat does not vary with temperature.

Line 16 is a parameter "NDEN" that is used to specify the density profile of the released material. For NDEN=0, the release is assumed to be an ideal gas with specific heat at constant pressure 1500J/kg/K. Water condensation effects are taken into account.

Line 17 is the mass rate of release, 7.69 kg/s.

Table C-3. SLAB Input - Horizonal Wellhead Release

| Value | Parameter | Line No. |
|---|---|---|
| 2 (horizontal), 3 (vertical) | IDSPL | 1 |
| 1 | NCALC | 2 |
| 0.0289 | WMS | 3 |
| 1500. | CPS | 4 |
| 111.50 | TBP | 5 |
| 0.0 | CMEDO | 6 |
| 509,880. | DHE | 7 |
| 3,349. | CPSL | 8 |
| 1,000. | RHOSL | 9 |
| -1.0 | SPB | 10 |
| 0.0 | SPC | 11 |
| 273. | TS | 12 |
| 7.69 | QS | 13 |
| $1.93 \times 10^{-2}$ | AS | 14 |
| 3,600. | TSD | 15 |
| 0. | QTIS | 16 |
| 5. | HS | 17 |
| 3,600. | TAV | 18 |
| 20,000. | XFFM | 19 |
| 1.6 | ZP(1) | 20 |
| 0. | ZP(2) | 21 |
| 0. | ZP(3) | 22 |
| 0. | ZP(4) | 23 |
| 0.1 | ZO | 24 |
| 10. | ZA | 25 |
| 1.5 | UA | 26 |
| 273. | TA | 27 |
| 75. | RH | 28 |
| F | STAB | 29 |
| -1. | TER | 30 |

## Table C-4. Partial SLAB Output

Time Averaged (TAV = 3,600 s) Volume Concentration: Concentration in the z = 1.60 Plane.

| Downwind Distance x (m) | Time of Max Conc (s) | Cloud Duration (s) | Effective Half Width bbc (m) | Average Concentration (Volume Fraction) at (x,y,z), y/bbc = 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
|---|---|---|---|---|---|---|---|---|---|
| 1.00 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $6.95 \times 10^{-2}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.02 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $7.73 \times 10^{-2}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.05 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $8.71 \times 10^{-2}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.08 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $9.92 \times 10^{-2}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.13 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.14 \times 10^{-1}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.18 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.32 \times 10^{-1}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.24 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.54 \times 10^{-1}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.32 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.81 \times 10^{-1}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.41 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $2.14 \times 10^{-1}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.52 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $2.54 \times 10^{-1}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.66 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $3.03 \times 10^{-1}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.84 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $3.63 \times 10^{-1}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2.05 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $4.36 \times 10^{-1}$ | $8.35 \times 10^{-41}$ | $5.76 \times 10^{-41}$ | $1.88 \times 10^{-41}$ | $2.84 \times 10^{-42}$ | $1.93 \times 10^{-43}$ | $5.61 \times 10^{-45}$ |
| 2.31 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $5.24 \times 10^{-1}$ | $1.18 \times 10^{-28}$ | $8.13 \times 10^{-29}$ | $2.65 \times 10^{-29}$ | $4.02 \times 10^{-30}$ | $2.79 \times 10^{-31}$ | $8.76 \times 10^{-33}$ |
| 2.63 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $6.31 \times 10^{-1}$ | $3.14 \times 10^{-20}$ | $2.16 \times 10^{-20}$ | $7.03 \times 10^{-21}$ | $1.07 \times 10^{-21}$ | $7.54 \times 10^{-23}$ | $2.42 \times 10^{-24}$ |
| 3.01 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $7.61 \times 10^{-1}$ | $1.99 \times 10^{-14}$ | $1.37 \times 10^{-14}$ | $4.45 \times 10^{-15}$ | $6.79 \times 10^{-16}$ | $4.82 \times 10^{-17}$ | $1.58 \times 10^{-18}$ |
| 3.49 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | $9.17 \times 10^{-1}$ | $1.86 \times 10^{-10}$ | $1.28 \times 10^{-10}$ | $4.16 \times 10^{-11}$ | $6.36 \times 10^{-12}$ | $4.55 \times 10^{-13}$ | $1.51 \times 10^{-14}$ |
| 4.08 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | 1.10 | $9.38 \times 10^{-8}$ | $6.45 \times 10^{-8}$ | $2.10 \times 10^{-8}$ | $3.21 \times 10^{-9}$ | $2.30 \times 10^{-10}$ | $7.71 \times 10^{-12}$ |
| 4.79 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | 1.33 | $6.31 \times 10^{-6}$ | $4.34 \times 10^{-6}$ | $1.41 \times 10^{-6}$ | $2.16 \times 10^{-7}$ | $1.55 \times 10^{-8}$ | $5.23 \times 10^{-10}$ |
| 5.67 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | 1.60 | $1.07 \times 10^{-4}$ | $7.33 \times 10^{-5}$ | $2.38 \times 10^{-5}$ | $3.65 \times 10^{-6}$ | $2.63 \times 10^{-7}$ | $8.89 \times 10^{-9}$ |
| 6.75 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | 1.91 | $6.99 \times 10^{-4}$ | $4.81 \times 10^{-4}$ | $1.56 \times 10^{-4}$ | $2.39 \times 10^{-5}$ | $1.73 \times 10^{-6}$ | $5.87 \times 10^{-8}$ |
| 8.07 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | 2.28 | $2.41 \times 10^{-3}$ | $1.66 \times 10^{-3}$ | $5.37 \times 10^{-4}$ | $8.24 \times 10^{-5}$ | $5.95 \times 10^{-6}$ | $2.03 \times 10^{-7}$ |
| 9.68 | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | 2.72 | $5.35 \times 10^{-3}$ | $3.68 \times 10^{-3}$ | $1.19 \times 10^{-3}$ | $1.83 \times 10^{-4}$ | $1.32 \times 10^{-5}$ | $4.51 \times 10^{-7}$ |
| $1.17 \times 10^{1}$ | $1.80 \times 10^{3}$ | $3.60 \times 10^{3}$ | 3.22 | $8.87 \times 10^{-3}$ | $6.09 \times 10^{-3}$ | $1.98 \times 10^{-3}$ | $3.03 \times 10^{-4}$ | $2.19 \times 10^{-5}$ | $7.48 \times 10^{-7}$ |
| $1.41 \times 10^{1}$ | $1.81 \times 10^{3}$ | $3.60 \times 10^{3}$ | 3.79 | $1.22 \times 10^{-2}$ | $8.38 \times 10^{-3}$ | $2.72 \times 10^{-3}$ | $4.17 \times 10^{-4}$ | $3.02 \times 10^{-5}$ | $1.03 \times 10^{-6}$ |
| $1.71 \times 10^{1}$ | $1.81 \times 10^{3}$ | $3.60 \times 10^{3}$ | 4.43 | $1.49 \times 10^{-2}$ | $1.03 \times 10^{-2}$ | $3.33 \times 10^{-3}$ | $5.11 \times 10^{-4}$ | $3.70 \times 10^{-5}$ | $1.27 \times 10^{-6}$ |
| $2.07 \times 10^{1}$ | $1.81 \times 10^{3}$ | $3.60 \times 10^{3}$ | 5.11 | $1.69 \times 10^{-2}$ | $1.16 \times 10^{-2}$ | $3.78 \times 10^{-3}$ | $5.80 \times 10^{-4}$ | $4.20 \times 10^{-5}$ | $1.43 \times 10^{-6}$ |
| $2.52 \times 10^{1}$ | $1.81 \times 10^{3}$ | $3.60 \times 10^{3}$ | 5.79 | $1.82 \times 10^{-2}$ | $1.25 \times 10^{-2}$ | $4.05 \times 10^{-3}$ | $6.22 \times 10^{-4}$ | $4.50 \times 10^{-5}$ | $1.54 \times 10^{-6}$ |
| $3.07 \times 10^{1}$ | $1.81 \times 10^{3}$ | $3.60 \times 10^{3}$ | 6.55 | $1.84 \times 10^{-2}$ | $1.27 \times 10^{-2}$ | $4.11 \times 10^{-3}$ | $6.31 \times 10^{-4}$ | $4.57 \times 10^{-5}$ | $1.57 \times 10^{-6}$ |

(continued)

Table C-4 (cont)

Time Averaged (TAV = 3,600 s) Volume Concentration: Concentration in the z = 1.60 Plane.

| Downwind Distance x (m) | Time of Max Conc (s) | Cloud Duration (s) | Effective Half Width bbc (m) | Average Concentration (Volume Fraction) at (x,y,z), y/bbc = 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
|---|---|---|---|---|---|---|---|---|---|
| $3.74 \times 10^{1}$ | $1.82 \times 10^{3}$ | $3.60 \times 10^{3}$ | 7.42 | $1.80 \times 10^{-2}$ | $1.23 \times 10^{-2}$ | $4.01 \times 10^{-3}$ | $6.15 \times 10^{-4}$ | $4.45 \times 10^{-5}$ | $1.52 \times 10^{-6}$ |
| $4.56 \times 10^{1}$ | $1.82 \times 10^{3}$ | $3.60 \times 10^{3}$ | 8.39 | $1.70 \times 10^{-2}$ | $1.17 \times 10^{-2}$ | $3.78 \times 10^{-3}$ | $5.80 \times 10^{-4}$ | $4.20 \times 10^{-5}$ | $1.43 \times 10^{-6}$ |
| $5.57 \times 10^{1}$ | $1.83 \times 10^{3}$ | $3.60 \times 10^{3}$ | 9.47 | $1.56 \times 10^{-2}$ | $1.08 \times 10^{-2}$ | $3.49 \times 10^{-3}$ | $5.35 \times 10^{-4}$ | $3.88 \times 10^{-5}$ | $1.33 \times 10^{-6}$ |
| $6.81 \times 10^{1}$ | $1.83 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.07 \times 10^{1}$ | $1.42 \times 10^{-2}$ | $9.77 \times 10^{-3}$ | $3.17 \times 10^{-3}$ | $4.86 \times 10^{-4}$ | $3.52 \times 10^{-5}$ | $1.20 \times 10^{-6}$ |
| $8.32 \times 10^{1}$ | $1.84 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.20 \times 10^{1}$ | $1.28 \times 10^{-2}$ | $8.77 \times 10^{-3}$ | $2.85 \times 10^{-3}$ | $4.37 \times 10^{-4}$ | $3.16 \times 10^{-5}$ | $1.08 \times 10^{-6}$ |
| $1.02 \times 10^{2}$ | $1.85 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.34 \times 10^{1}$ | $1.14 \times 10^{-2}$ | $7.81 \times 10^{-3}$ | $2.53 \times 10^{-3}$ | $3.89 \times 10^{-4}$ | $2.82 \times 10^{-5}$ | $9.63 \times 10^{-7}$ |
| $1.25 \times 10^{2}$ | $1.86 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.51 \times 10^{1}$ | $1.00 \times 10^{-2}$ | $6.90 \times 10^{-3}$ | $2.24 \times 10^{-3}$ | $3.43 \times 10^{-4}$ | $2.49 \times 10^{-5}$ | $8.50 \times 10^{-7}$ |
| $1.52 \times 10^{2}$ | $1.87 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.70 \times 10^{1}$ | $8.80 \times 10^{-3}$ | $6.05 \times 10^{-3}$ | $1.96 \times 10^{-3}$ | $3.01 \times 10^{-4}$ | $2.18 \times 10^{-5}$ | $7.44 \times 10^{-7}$ |
| $1.87 \times 10^{2}$ | $1.89 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.92 \times 10^{1}$ | $7.68 \times 10^{-3}$ | $5.28 \times 10^{-3}$ | $1.71 \times 10^{-3}$ | $2.63 \times 10^{-4}$ | $1.90 \times 10^{-5}$ | $6.51 \times 10^{-7}$ |
| $2.29 \times 10^{2}$ | $1.90 \times 10^{3}$ | $3.60 \times 10^{3}$ | $2.18 \times 10^{1}$ | $6.65 \times 10^{-3}$ | $4.57 \times 10^{-3}$ | $1.48 \times 10^{-3}$ | $2.27 \times 10^{-4}$ | $1.65 \times 10^{-5}$ | $5.61 \times 10^{-7}$ |
| $2.80 \times 10^{2}$ | $1.93 \times 10^{3}$ | $3.60 \times 10^{3}$ | $2.49 \times 10^{1}$ | $5.71 \times 10^{-3}$ | $3.92 \times 10^{-3}$ | $1.27 \times 10^{-3}$ | $1.95 \times 10^{-4}$ | $1.41 \times 10^{-5}$ | $4.84 \times 10^{-7}$ |
| $3.43 \times 10^{2}$ | $1.96 \times 10^{3}$ | $3.60 \times 10^{3}$ | $2.87 \times 10^{1}$ | $4.85 \times 10^{-3}$ | $3.33 \times 10^{-3}$ | $1.08 \times 10^{-3}$ | $1.66 \times 10^{-4}$ | $1.20 \times 10^{-5}$ | $4.10 \times 10^{-7}$ |
| $4.20 \times 10^{2}$ | $1.99 \times 10^{3}$ | $3.60 \times 10^{3}$ | $3.33 \times 10^{1}$ | $4.07 \times 10^{-3}$ | $2.80 \times 10^{-3}$ | $9.09 \times 10^{-4}$ | $1.39 \times 10^{-4}$ | $1.01 \times 10^{-5}$ | $3.44 \times 10^{-7}$ |
| $5.15 \times 10^{2}$ | $2.04 \times 10^{3}$ | $3.60 \times 10^{3}$ | $3.91 \times 10^{1}$ | $3.38 \times 10^{-3}$ | $2.32 \times 10^{-3}$ | $7.55 \times 10^{-4}$ | $1.16 \times 10^{-4}$ | $8.38 \times 10^{-6}$ | $2.89 \times 10^{-7}$ |
| $6.30 \times 10^{2}$ | $2.09 \times 10^{3}$ | $3.60 \times 10^{3}$ | $4.65 \times 10^{1}$ | $2.77 \times 10^{-3}$ | $1.91 \times 10^{-3}$ | $6.19 \times 10^{-4}$ | $9.49 \times 10^{-5}$ | $6.87 \times 10^{-6}$ | $2.36 \times 10^{-7}$ |
| $7.73 \times 10^{2}$ | $2.15 \times 10^{3}$ | $3.60 \times 10^{3}$ | $5.58 \times 10^{1}$ | $2.25 \times 10^{-3}$ | $1.54 \times 10^{-3}$ | $5.01 \times 10^{-4}$ | $7.68 \times 10^{-5}$ | $5.57 \times 10^{-6}$ | $1.90 \times 10^{-7}$ |
| $9.47 \times 10^{2}$ | $2.23 \times 10^{3}$ | $3.60 \times 10^{3}$ | $6.75 \times 10^{1}$ | $1.80 \times 10^{-3}$ | $1.24 \times 10^{-3}$ | $4.02 \times 10^{-4}$ | $6.16 \times 10^{-5}$ | $4.46 \times 10^{-6}$ | $1.49 \times 10^{-7}$ |
| $1.16 \times 10^{3}$ | $2.33 \times 10^{3}$ | $3.60 \times 10^{3}$ | $8.23 \times 10^{1}$ | $1.43 \times 10^{-3}$ | $9.82 \times 10^{-4}$ | $3.19 \times 10^{-4}$ | $4.89 \times 10^{-5}$ | $3.54 \times 10^{-6}$ | $1.22 \times 10^{-7}$ |
| $1.42 \times 10^{3}$ | $2.45 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.01 \times 10^{2}$ | $1.12 \times 10^{-3}$ | $7.73 \times 10^{-4}$ | $2.51 \times 10^{-4}$ | $3.85 \times 10^{-5}$ | $2.79 \times 10^{-6}$ | $9.64 \times 10^{-8}$ |
| $1.74 \times 10^{3}$ | $2.60 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.24 \times 10^{2}$ | $8.81 \times 10^{-4}$ | $6.05 \times 10^{-4}$ | $1.96 \times 10^{-4}$ | $3.01 \times 10^{-5}$ | $2.18 \times 10^{-6}$ | $7.55 \times 10^{-8}$ |
| $2.13 \times 10^{3}$ | $2.78 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.52 \times 10^{2}$ | $6.86 \times 10^{-4}$ | $4.71 \times 10^{-4}$ | $1.53 \times 10^{-4}$ | $2.35 \times 10^{-5}$ | $1.70 \times 10^{-6}$ | $5.91 \times 10^{-8}$ |
| $2.62 \times 10^{3}$ | $3.00 \times 10^{3}$ | $3.60 \times 10^{3}$ | $1.86 \times 10^{2}$ | $5.31 \times 10^{-4}$ | $3.65 \times 10^{-4}$ | $1.19 \times 10^{-4}$ | $1.82 \times 10^{-5}$ | $1.32 \times 10^{-6}$ | $4.37 \times 10^{-8}$ |
| $3.21 \times 10^{3}$ | $3.27 \times 10^{3}$ | $3.60 \times 10^{3}$ | $2.28 \times 10^{2}$ | $4.08 \times 10^{-4}$ | $2.81 \times 10^{-4}$ | $9.11 \times 10^{-5}$ | $1.40 \times 10^{-5}$ | $1.01 \times 10^{-6}$ | $3.52 \times 10^{-8}$ |
| $3.93 \times 10^{3}$ | $3.60 \times 10^{3}$ | $3.60 \times 10^{3}$ | $2.77 \times 10^{2}$ | $3.09 \times 10^{-4}$ | $2.13 \times 10^{-4}$ | $6.90 \times 10^{-5}$ | $1.06 \times 10^{-5}$ | $7.65 \times 10^{-7}$ | $2.70 \times 10^{-8}$ |
| $4.82 \times 10^{3}$ | $4.10 \times 10^{3}$ | $3.87 \times 10^{3}$ | $3.40 \times 10^{2}$ | $2.05 \times 10^{-4}$ | $1.41 \times 10^{-4}$ | $4.58 \times 10^{-5}$ | $7.02 \times 10^{-6}$ | $5.10 \times 10^{-7}$ | $1.76 \times 10^{-8}$ |
| $5.94 \times 10^{3}$ | $4.70 \times 10^{3}$ | $4.19 \times 10^{3}$ | $4.14 \times 10^{2}$ | $1.39 \times 10^{-4}$ | $9.57 \times 10^{-5}$ | $3.11 \times 10^{-5}$ | $4.77 \times 10^{-6}$ | $3.46 \times 10^{-7}$ | $1.09 \times 10^{-8}$ |
| $7.33 \times 10^{3}$ | $5.45 \times 10^{3}$ | $4.58 \times 10^{3}$ | $5.02 \times 10^{2}$ | $9.06 \times 10^{-5}$ | $6.22 \times 10^{-5}$ | $2.02 \times 10^{-5}$ | $3.10 \times 10^{-6}$ | $2.24 \times 10^{-7}$ | $8.61 \times 10^{-9}$ |
| $9.07 \times 10^{3}$ | $6.36 \times 10^{3}$ | $5.03 \times 10^{3}$ | $6.05 \times 10^{2}$ | $5.27 \times 10^{-5}$ | $3.62 \times 10^{-5}$ | $1.17 \times 10^{-5}$ | $1.80 \times 10^{-6}$ | $1.31 \times 10^{-7}$ | $4.02 \times 10^{-9}$ |
| $1.13 \times 10^{4}$ | $7.47 \times 10^{3}$ | $5.57 \times 10^{3}$ | $7.23 \times 10^{2}$ | $2.55 \times 10^{-5}$ | $1.75 \times 10^{-5}$ | $5.70 \times 10^{-6}$ | $8.73 \times 10^{-7}$ | $6.35 \times 10^{-8}$ | $2.33 \times 10^{-9}$ |
| $1.40 \times 10^{4}$ | $8.84 \times 10^{3}$ | $6.11 \times 10^{3}$ | $8.62 \times 10^{2}$ | $1.46 \times 10^{-5}$ | $1.00 \times 10^{-5}$ | $3.26 \times 10^{-6}$ | $5.00 \times 10^{-7}$ | $3.61 \times 10^{-8}$ | $1.59 \times 10^{-9}$ |
| $1.75 \times 10^{4}$ | $1.05 \times 10^{4}$ | $6.67 \times 10^{3}$ | $1.02 \times 10^{3}$ | $1.04 \times 10^{-5}$ | $7.13 \times 10^{-6}$ | $2.31 \times 10^{-6}$ | $3.55 \times 10^{-7}$ | $2.58 \times 10^{-8}$ | $6.70 \times 10^{-10}$ |
| $2.20 \times 10^{4}$ | $1.26 \times 10^{4}$ | $7.49 \times 10^{3}$ | $1.21 \times 10^{3}$ | $5.98 \times 10^{-6}$ | $4.11 \times 10^{-6}$ | $1.33 \times 10^{-6}$ | $2.05 \times 10^{-7}$ | $1.48 \times 10^{-8}$ | $4.55 \times 10^{-10}$ |

Table C-5. Input for DEGADIS Simulation of a Vertical Wellhead Release

| Value | | | Line Number |
|---|---|---|---|
| Release from a | | | 1 |
| Well Head: | | | 2 |
| Verticle Jet Simulation | | | 3 |
| | | | 4 |
| 1.5 | 10. | | 5 |
| 0.1 | | | 6 |
| 1 | 6 | 0. | 7 |
| 273. | 1. | 75. | 8 |
| 273. | | | 9 |
| CPD | | | 10 |
| 25.2 | | | 11 |
| 3,600. | | | 12 |
| 273. | | | 13 |
| $4.7 \times 10^{-4}$ | $1.0 \times 10^{-4}$ | 1.6 | 14 |
| 0 | 1,500. | 0.0 | 15 |
| 0 | | | 16 |
| 7.69 | | | 17 |
| 5.0 | 0.0192 | | 18 |
| 3,600. | | | 19 |
| 50. | | | 20 |

Line 18 contains the height of release (5 m) and the effective diameter (0.0192 m)

Line 19 is the duration of release, 3,600 s.

Line 20 is the distance between points at which DEGADIS calculates the output.

**DEGADIS Output**

A partial DEGADIS output is given in Table C-6. The first column gives the distance downwind and the second gives the elevation. As can be seen, the plume rises substantially because of its initial momentum. The third column gives the concentration of the released gas as a mole fraction, the fourth column gives the concentration in $kg/m^3$ and the fifth column gives the density in $kg/m^3$. As can be seen, the density rapidly approaches that of the surrounding air, 1.29 $kg/m^3$. The fifth column gives the temperature of the plume, which remains constant at 273K because the released plume and the air both have that temperature. The sixth column gives the plume horizontal standard deviation, $\sigma$ y, and the seventh column gives the vertical standard deviation, $\sigma$ z (the concentration across the plume is approximated by a Gaussian distribution in DEGADIS). As can be seen, at a height of 1.60 m, the predicted width of the plume (the distance across the wind to the upper or lower Levels of Concern, $LC_{01}$ and ERPG-2) is zero so that LOCs are not predicted to be seen at ground level. This is a typical result for vertical jets of sour gas in stable weather conditions, whether DEGADIS, SLAB, or SAPLUME is used.

## SAMPLE SAPLUME CALCULATIONS

**SAPLUME Input**

Table C-7 contains the input for the model SAPLUME corresponding to Table C-3, which contains the SLAB input for a horizontal release with composition D. The first few lines of input begin with four asterisks and are title cards, followed by a blank which tells the code that the titles have ended. Each subsequent line or group of lines begins with a keyword, followed by numbers in exponential notation to three significant figures.

"SITE" tells SAPLUME that there is a site with one radius and one sector (this is the default when the model is not considering a real site). The following line gives the one radius, arbitrarily set at 10,000 m, with one person arbitrarily set at that point (in the mode of operation chosen for the current problem, the model ignores these numbers).

"WEATHER" specifies that one weather condition only, category F, is being considered (because the 1.000 that follows WEATHER begins at space 61. For E the space would be 51, for D, 41 and so forth. The model can consider all six weather categories at once with up to four velocity subdivisions in each.) In this case, there is one velocity subdivision, specified as 1.5 m/s (first line after weather), and the probability that the wind blows into the one sector is unity (second line after weather).

## Table C-6. Partial DEGADIS Output - Vertical Jet

## Release from a Wellhead Pipeline Vertical Jet Simulation

| Downwind Distance (m) | Elevation (m) | Mole Fraction | Concentration ($kg/m^3$) | Density ($kg/m^3$) | Temperature (K) | σ y (m) | σ z (m) | At z = 1.60 m: Mole Fraction | At z = 1.60 m: Width to mol%: $1.00 \times 10^{-2}$ (m) | At z = 1.60 m: Width to mol%: $4.70 \times 10^{-2}$ (m) | Maximum Mole Fraction | Elevation for Maximum Mole Fraction (m) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $1.000 \times 10^{-30}$ | 5.15 | 1.00 | 1.13 | 1.13 | 273 | $1.060 \times 10^{-2}$ | $1.059 \times 10^{-2}$ | .000 | 0 | 0 | 1.00 | 5.15 |
| 29.5 | 120. | $8.279 \times 10^{-4}$ | $9.314 \times 10^{-4}$ | 1.29 | 273 | 12.1 | 12.0 | .000 | 0 | 0 | $8.279 \times 10^{-4}$ | 120. |
| 61.4 | 142. | $5.644 \times 10^{-4}$ | $6.349 \times 10^{-4}$ | 1.29 | 273 | 16.0 | 15.6 | .000 | 0 | 0 | $5.644 \times 10^{-4}$ | 142. |
| 99.6 | 157. | $4.254 \times 10^{-4}$ | $4.786 \times 10^{-4}$ | 1.29 | 273 | 19.2 | 18.4 | .000 | 0 | 0 | $4.254 \times 10^{-4}$ | 157. |
| 142. | 169. | $3.425 \times 10^{-4}$ | $3.853 \times 10^{-4}$ | 1.29 | 273 | 22.0 | 20.6 | .000 | 0 | 0 | $3.425 \times 10^{-4}$ | 169. |
| 191. | 180. | $2.832 \times 10^{-4}$ | $3.186 \times 10^{-4}$ | 1.29 | 273 | 24.6 | 22.4 | .000 | 0 | 0 | $2.832 \times 10^{-4}$ | 180. |
| 240. | 188. | $2.432 \times 10^{-4}$ | $2.736 \times 10^{-4}$ | 1.29 | 273 | 27.0 | 23.9 | .000 | 0 | 0 | $2.432 \times 10^{-4}$ | 188. |
| 289. | 196. | $2.140 \times 10^{-4}$ | $2.408 \times 10^{-4}$ | 1.29 | 273 | 29.2 | 25.2 | .000 | 0 | 0 | $2.140 \times 10^{-4}$ | 196. |
| 339. | 202. | $1.915 \times 10^{-4}$ | $2.155 \times 10^{-4}$ | 1.29 | 273 | 31.4 | 26.3 | .000 | 0 | 0 | $1.915 \times 10^{-4}$ | 202. |
| 389. | 207. | $1.736 \times 10^{-4}$ | $1.953 \times 10^{-4}$ | 1.29 | 273 | 33.4 | 27.2 | .000 | 0 | 0 | $1.736 \times 10^{-4}$ | 207. |
| 438. | 212. | $1.588 \times 10^{-4}$ | $1.786 \times 10^{-4}$ | 1.29 | 273 | 35.5 | 28.1 | .000 | 0 | 0 | $1.588 \times 10^{-4}$ | 212. |
| 488. | 217. | $1.464 \times 10^{-4}$ | $1.647 \times 10^{-4}$ | 1.29 | 273 | 37.5 | 28.8 | .000 | 0 | 0 | $1.464 \times 10^{-4}$ | 217. |
| 538. | 221. | $1.358 \times 10^{-4}$ | $1.528 \times 10^{-4}$ | 1.29 | 273 | 39.4 | 29.5 | .000 | 0 | 0 | $1.358 \times 10^{-4}$ | 221. |
| 588. | 225. | $1.267 \times 10^{-4}$ | $1.425 \times 10^{-4}$ | 1.29 | 273 | 41.4 | 30.1 | .000 | 0 | 0 | $1.267 \times 10^{-4}$ | 225. |
| 638. | 228. | $1.187 \times 10^{-4}$ | $1.335 \times 10^{-4}$ | 1.29 | 273 | 43.4 | 30.7 | .000 | 0 | 0 | $1.187 \times 10^{-4}$ | 228. |
| 688. | 231. | $1.116 \times 10^{-4}$ | $1.255 \times 10^{-4}$ | 1.29 | 273 | 45.3 | 31.2 | .000 | 0 | 0 | $1.116 \times 10^{-4}$ | 231. |
| 738. | 234. | $1.053 \times 10^{-4}$ | $1.185 \times 10^{-4}$ | 1.29 | 273 | 47.2 | 31.7 | .000 | 0 | 0 | $1.053 \times 10^{-4}$ | 234. |
| 787. | 237. | $9.965 \times 10^{-5}$ | $1.121 \times 10^{-4}$ | 1.29 | 273 | 49.2 | 32.2 | .000 | 0 | 0 | $9.965 \times 10^{-5}$ | 237. |
| 837. | 240. | $9.457 \times 10^{-5}$ | $1.064 \times 10^{-4}$ | 1.29 | 273 | 51.1 | 32.6 | .000 | 0 | 0 | $9.457 \times 10^{-5}$ | 240. |
| 887. | 242. | $8.995 \times 10^{-5}$ | $1.012 \times 10^{-4}$ | 1.29 | 273 | 53.1 | 33.0 | .000 | 0 | 0 | $8.995 \times 10^{-5}$ | 242. |
| 937. | 245. | $8.575 \times 10^{-5}$ | $9.647 \times 10^{-5}$ | 1.29 | 273 | 55.0 | 33.4 | .000 | 0 | 0 | $8.575 \times 10^{-5}$ | 245. |
| 987. | 247. | $8.190 \times 10^{-5}$ | $9.213 \times 10^{-5}$ | 1.29 | 273 | 56.9 | 33.8 | .000 | 0 | 0 | $8.190 \times 10^{-5}$ | 247. |
| $1.037 \times 10^{3}$ | 249. | $7.836 \times 10^{-5}$ | $8.816 \times 10^{-5}$ | 1.29 | 273 | 58.9 | 34.2 | .000 | 0 | 0 | $7.836 \times 10^{-5}$ | 249. |
| $1.087 \times 10^{3}$ | 251. | $7.510 \times 10^{-5}$ | $8.449 \times 10^{-5}$ | 1.29 | 273 | 60.8 | 34.5 | .000 | 0 | 0 | $7.510 \times 10^{-5}$ | 251. |
| $1.137 \times 10^{3}$ | 253. | $7.208 \times 10^{-5}$ | $8.109 \times 10^{-5}$ | 1.29 | 273 | 62.7 | 34.9 | .000 | 0 | 0 | $7.208 \times 10^{-5}$ | 253. |
| $1.187 \times 10^{3}$ | 255. | $6.928 \times 10^{-5}$ | $7.794 \times 10^{-5}$ | 1.29 | 273 | 64.7 | 35.2 | .000 | 0 | 0 | $6.928 \times 10^{-5}$ | 255. |
| $1.237 \times 10^{3}$ | 257. | $6.667 \times 10^{-5}$ | $7.501 \times 10^{-5}$ | 1.29 | 273 | 66.6 | 35.5 | .000 | 0 | 0 | $6.667 \times 10^{-5}$ | 257. |
| $1.287 \times 10^{3}$ | 258. | $6.424 \times 10^{-5}$ | $7.228 \times 10^{-5}$ | 1.29 | 273 | 68.5 | 35.8 | .000 | 0 | 0 | $6.424 \times 10^{-5}$ | 258. |
| $1.337 \times 10^{3}$ | 260. | $6.197 \times 10^{-5}$ | $6.972 \times 10^{-5}$ | 1.29 | 273 | 70.5 | 36.1 | .000 | 0 | 0 | $6.197 \times 10^{-5}$ | 260. |
| $1.387 \times 10^{3}$ | 262. | $5.984 \times 10^{-5}$ | $6.733 \times 10^{-5}$ | 1.29 | 273 | 72.4 | 36.3 | .000 | 0 | 0 | $5.984 \times 10^{-5}$ | 262. |

## Table C-6 (cont)

| Downwind Distance (m) | Elevation (m) | Mole Fraction | Concentration (kg/m³) | Density (kg/m³) | Temperature (K) | σ y (m) | σ z (m) | At z = 1.60 m: Mole Fraction | Width to mol%: $1.00 \times 10^{-2}$ (m) | Width to mol%: $4.70 \times 10^{-2}$ (m) | Maximum Mole Fraction | Elevation for Maximum Mole Fraction (m) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $1.437 \times 10^{3}$ | 263. | $5.785 \times 10^{-5}$ | $6.508 \times 10^{-5}$ | 1.29 | 273 | 74.3 | 36.6 | .000 | 0 | 0 | $5.785 \times 10^{-5}$ | 263. |
| $1.487 \times 10^{3}$ | 265. | $5.597 \times 10^{-5}$ | $6.296 \times 10^{-5}$ | 1.29 | 273 | 76.3 | 36.9 | .000 | 0 | 0 | $5.597 \times 10^{-5}$ | 265. |
| $1.537 \times 10^{3}$ | 266. | $5.420 \times 10^{-5}$ | $6.097 \times 10^{-5}$ | 1.29 | 273 | 78.2 | 37.1 | .000 | 0 | 0 | $5.420 \times 10^{-5}$ | 266. |
| $1.587 \times 10^{3}$ | 267. | $5.253 \times 10^{-5}$ | $5.909 \times 10^{-5}$ | 1.29 | 273 | 80.2 | 37.4 | .000 | 0 | 0 | $5.253 \times 10^{-5}$ | 267. |
| $1.637 \times 10^{3}$ | 269. | $5.095 \times 10^{-5}$ | $5.732 \times 10^{-5}$ | 1.29 | 273 | 82.1 | 37.6 | .000 | 0 | 0 | $5.095 \times 10^{-5}$ | 269. |
| $1.687 \times 10^{3}$ | 270. | $4.945 \times 10^{-5}$ | $5.564 \times 10^{-5}$ | 1.29 | 273 | 84.0 | 37.9 | .000 | 0 | 0 | $4.945 \times 10^{-5}$ | 270. |
| $1.737 \times 10^{3}$ | 271. | $4.804 \times 10^{-5}$ | $5.404 \times 10^{-5}$ | 1.29 | 273 | 85.9 | 38.1 | .000 | 0 | 0 | $4.804 \times 10^{-5}$ | 271. |
| $1.787 \times 10^{3}$ | 273. | $4.669 \times 10^{-5}$ | $5.253 \times 10^{-5}$ | 1.29 | 273 | 87.9 | 38.3 | .000 | 0 | 0 | $4.669 \times 10^{-5}$ | 273. |
| $1.837 \times 10^{3}$ | 274. | $4.542 \times 10^{-5}$ | $5.109 \times 10^{-5}$ | 1.29 | 273 | 89.8 | 38.6 | .000 | 0 | 0 | $4.542 \times 10^{-5}$ | 274. |
| $1.887 \times 10^{3}$ | 275. | $4.420 \times 10^{-5}$ | $4.973 \times 10^{-5}$ | 1.29 | 273 | 91.7 | 38.8 | .000 | 0 | 0 | $4.420 \times 10^{-5}$ | 275. |
| $1.937 \times 10^{3}$ | 276. | $4.304 \times 10^{-5}$ | $4.842 \times 10^{-5}$ | 1.29 | 273 | 93.7 | 39.0 | .000 | 0 | 0 | $4.304 \times 10^{-5}$ | 276. |
| $1.987 \times 10^{3}$ | 277. | $4.194 \times 10^{-5}$ | $4.718 \times 10^{-5}$ | 1.29 | 273 | 95.6 | 39.2 | .000 | 0 | 0 | $4.194 \times 10^{-5}$ | 277. |
| $2.037 \times 10^{3}$ | 278. | $4.089 \times 10^{-5}$ | $4.600 \times 10^{-5}$ | 1.29 | 273 | 97.5 | 39.5 | .000 | 0 | 0 | $4.089 \times 10^{-5}$ | 278. |
| $2.087 \times 10^{3}$ | 279. | $3.988 \times 10^{-5}$ | $4.487 \times 10^{-5}$ | 1.29 | 273 | 99.4 | 39.7 | .000 | 0 | 0 | $3.988 \times 10^{-5}$ | 279. |
| $2.137 \times 10^{3}$ | 280. | $3.892 \times 10^{-5}$ | $4.378 \times 10^{-5}$ | 1.29 | 273 | 101. | 39.9 | .000 | 0 | 0 | $3.892 \times 10^{-5}$ | 280. |
| $2.187 \times 10^{3}$ | 281. | $3.800 \times 10^{-5}$ | $4.275 \times 10^{-5}$ | 1.29 | 273 | 103. | 40.1 | .000 | 0 | 0 | $3.800 \times 10^{-5}$ | 281. |
| $2.237 \times 10^{3}$ | 282. | $3.711 \times 10^{-5}$ | $4.175 \times 10^{-5}$ | 1.29 | 273 | 105. | 40.3 | .000 | 0 | 0 | $3.711 \times 10^{-5}$ | 282. |
| $2.287 \times 10^{3}$ | 283. | $3.627 \times 10^{-5}$ | $4.080 \times 10^{-5}$ | 1.29 | 273 | 107. | 40.5 | .000 | 0 | 0 | $3.627 \times 10^{-5}$ | 283. |
| $2.337 \times 10^{3}$ | 284. | $3.546 \times 10^{-5}$ | $3.989 \times 10^{-5}$ | 1.29 | 273 | 109. | 40.7 | .000 | 0 | 0 | $3.546 \times 10^{-5}$ | 284. |
| $2.387 \times 10^{3}$ | 285. | $3.468 \times 10^{-5}$ | $3.901 \times 10^{-5}$ | 1.29 | 273 | 111. | 40.9 | .000 | 0 | 0 | $3.468 \times 10^{-5}$ | 285. |
| $2.437 \times 10^{3}$ | 286. | $3.393 \times 10^{-5}$ | $3.817 \times 10^{-5}$ | 1.29 | 273 | 113. | 41.1 | .000 | 0 | 0 | $3.393 \times 10^{-5}$ | 286. |
| $2.487 \times 10^{3}$ | 286. | $3.321 \times 10^{-5}$ | $3.736 \times 10^{-5}$ | 1.29 | 273 | 115. | 41.2 | .000 | 0 | 0 | $3.321 \times 10^{-5}$ | 286. |
| $2.537 \times 10^{3}$ | 287. | $3.252 \times 10^{-5}$ | $3.658 \times 10^{-5}$ | 1.29 | 273 | 117. | 41.4 | .000 | 0 | 0 | $3.252 \times 10^{-5}$ | 287. |
| $2.587 \times 10^{3}$ | 288. | $3.185 \times 10^{-5}$ | $3.583 \times 10^{-5}$ | 1.29 | 273 | 119. | 41.6 | .000 | 0 | 0 | $3.185 \times 10^{-5}$ | 288. |
| $2.637 \times 10^{3}$ | 289. | $3.121 \times 10^{-5}$ | $3.511 \times 10^{-5}$ | 1.29 | 273 | 120. | 41.8 | .000 | 0 | 0 | $3.121 \times 10^{-5}$ | 289. |
| $2.687 \times 10^{3}$ | 290. | $3.059 \times 10^{-5}$ | $3.441 \times 10^{-5}$ | 1.29 | 273 | 122. | 42.0 | .000 | 0 | 0 | $3.059 \times 10^{-5}$ | 290. |
| $2.737 \times 10^{3}$ | 290. | $2.999 \times 10^{-5}$ | $3.374 \times 10^{-5}$ | 1.29 | 273 | 124. | 42.2 | .000 | 0 | 0 | $2.999 \times 10^{-5}$ | 290. |
| $2.787 \times 10^{3}$ | 291. | $2.941 \times 10^{-5}$ | $3.309 \times 10^{-5}$ | 1.29 | 273 | 126. | 42.3 | .000 | 0 | 0 | $2.941 \times 10^{-5}$ | 291. |
| $2.837 \times 10^{3}$ | 292. | $2.885 \times 10^{-5}$ | $3.246 \times 10^{-5}$ | 1.29 | 273 | 128. | 42.5 | .000 | 0 | 0 | $2.885 \times 10^{-5}$ | 292. |
| $2.887 \times 10^{3}$ | 293. | $2.831 \times 10^{-5}$ | $3.185 \times 10^{-5}$ | 1.29 | 273 | 130. | 42.7 | .000 | 0 | 0 | $2.831 \times 10^{-5}$ | 293. |
| $2.937 \times 10^{3}$ | 293. | $2.779 \times 10^{-5}$ | $3.127 \times 10^{-5}$ | 1.29 | 273 | 132. | 42.9 | .000 | 0 | 0 | $2.779 \times 10^{-5}$ | 293. |
| $2.987 \times 10^{3}$ | 294. | $2.729 \times 10^{-5}$ | $3.070 \times 10^{-5}$ | 1.29 | 273 | 134. | 43.0 | .000 | 0 | 0 | $2.729 \times 10^{-5}$ | 294. |
| $3.037 \times 10^{3}$ | 295. | $2.680 \times 10^{-5}$ | $3.015 \times 10^{-5}$ | 1.29 | 273 | 136. | 43.2 | .000 | 0 | 0 | $2.680 \times 10^{-5}$ | 295. |

## Table C-6 (cont)

| Downwind Distance (m) | Elevation (m) | Mole Fraction | Concentration ($kg/m^3$) | Density ($kg/m^3$) | Temperature (K) | $\sigma y$ (m) | $\sigma z$ (m) | At x = 1.60 m: Mole Fraction | At x = 1.60 m: Width to mol%: $1.00 \times 10^{-2}$ (m) | At x = 1.60 m: Width to mol%: $4.70 \times 10^{-2}$ (m) | Maximum Mole Fraction | Elevation for Maximum Mole Fraction (m) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $3.087 \times 10^3$ | 295. | $2.633 \times 10^{-5}$ | $2.962 \times 10^{-5}$ | 1.29 | 273 | 138. | 43.4 | .000 | 0 | 0 | $2.633 \times 10^{-5}$ | 295. |
| $3.137 \times 10^3$ | 296. | $2.588 \times 10^{-5}$ | $2.911 \times 10^{-5}$ | 1.29 | 273 | 139. | 43.5 | .000 | 0 | 0 | $2.588 \times 10^{-5}$ | 296. |
| $3.187 \times 10^3$ | 297. | $2.543 \times 10^{-5}$ | $2.861 \times 10^{-5}$ | 1.29 | 273 | 141. | 43.7 | .000 | 0 | 0 | $2.543 \times 10^{-5}$ | 297. |
| $3.237 \times 10^3$ | 297. | $2.500 \times 10^{-5}$ | $2.813 \times 10^{-5}$ | 1.29 | 273 | 143. | 43.9 | .000 | 0 | 0 | $2.500 \times 10^{-5}$ | 297. |
| $3.262 \times 10^3$ | 297. | $2.479 \times 10^{-5}$ | $2.789 \times 10^{-5}$ | 1.29 | 273 | 144. | 43.9 | .000 | 0 | 0 | $2.479 \times 10^{-5}$ | 297. |

The entries on the "PROPERTIES" line are as follows: the ambient temperature is 273K at which temperature the density of air is 1.29 $kg/m^3$ and its specific heat at constant pressure is 990 J/kg/K. At a temperature of 273K, the density of the released gas is 1.141 $kg/m^3$ and the specific heat at constant pressure is 1,500 J/kg/K.

The entries on the "SOURCE" line are as follows: the rate of release is 7.69 $kg/m^3$ at a temperature of 273K. The amount of air initially entrained with the source is zero. The angle of release is zero radians (horizontal). The height of release is 5 m. The initial momentum flux is 2,540 kg m $s^{-2}$ and is the product of the rate of release and the exit velocity (the speed of sound is approximately 330 m/s).

The "INTERVAL" line specifies that SAPLUME calculations start at a downwind distance x of 0.1 m and that calculations are performed at a uniform spacing of 0.15 in $\log_{10}(x)$.

On the "ROUG" line, the surface roughness length is 0.1 m and the windspeed is measured at a height of 10 m.

The "HAZARD" line specifies two levels of concern. As explained above in the discussion of the SLAB results, these are the $LC_{01}$ of 470 ppm (approximately $5.32 \times 10^{-4}$ $kg/m^3$) and the ERPG-2 of 100 ppm ($1.141 \times 10^{-4}$ $kg/m^3$).

"VGRAD" informs SAPLUME that it should consider the velocity gradient and the temperature gradient in the atmosphere. SAPLUME uses standard textbook formulae for these gradients. If the first entry after VGRAD were zero, velocity would be constant as a function of height. Similarly, if the second entry after VGRAD were zero, the temperature of the atmosphere would remain constant as height increases.

A value of 3 after "NEUT" specifies one of three parametrizations for the standard deviations in the Gaussian model once the released material has evolved out of the jet phase. NEUT =3 corresponds to a parameterization that is appropriate for a rural site. "DUR" specifies that the duration of release is one hour.

Finally, the repetition of "END" terminates the run of SAPLUME.

## SAPLUME Output

A partial SAPLUME output corresponding to the input in Table C-7 is given in Table C-8. This table indicates that, for hazard level 1 (i.e., the $LC_{01}$ of 470 ppm discussed above) the plume touches down at a downwind distance of approximately 63 m and extends to about 3 km, covering an area of about $10^5$ $m^2$ (one tenth of a square kilometer). The table of pairs of values of downwind distance, x, and width can be coupled to a plotting routine to give contours of constant concentration. Similarly, hazard level 2 (the ERPG-2 of 100 ppm)

## Table C-7. Input for SAPLUME Runs

| | | | | | | |
|---|---|---|---|---|---|---|
| **** | EPA Hydrogen Sulfide Runs | | | | | |
| **** | January 1993 | | | | | |
| **** | No Protective Measures | | | | | |
| **** | Composition D - 30% $H_2S$ at Wellhead | | | | | |
| **** | $2x10^7$ SCFD: | | | | | |
| **** | Horizontal Release | | | | | |
| **** | $H_2S$ Release Rate - 3.073 kg/s | | | | | |
| **** | Total Mixture Release Rate - 7.69 kg/s | | | | | |
| **** | Hazard Level - ERPG-2 (100 ppm) and | | | | | |
| **** | $LC_{01}$ (470 ppm) Both Adjusted for Stream Composition | | | | | |
| **** | Category F Weather, Windspeed 1.5 m/s | | | | | |
| SITE | 1.000 | 1.000 | | | | |
| $1.000x10^4$ | | | | | | |
| 1.000 | | | | | | |
| WEATHER | 1.000 | | | | | |
| 1.500 | | | | | | |
| $2.500x10^{-1}$ | | | | | | |
| PROP | $2.730x10^2$ | 1.290 | $9.900x10^2$ | $2.730x10^2$ | 1.141 | $1.500x10^3$ |
| SOURCE | 7.690 | $2.730x10^2$ | 0.000 | 0.000 | 5.000 | $2.540x10^3$ |
| INTERVAL | $1.000x10^{-1}$ | $1.5000x10^{-1}$ | | | | |
| ROUG | $1.0000x10^{-1}$ | $1.000x10^1$ | | | | |
| HAZARD | 2.000 | | | | | |
| $5.320x10^{-4}$ | $1.141x10^{-4}$ | | | | | |
| VGRAD | 1.000 | 1.000 | | | | |
| NEUT | 3.000 | | | | | |
| DUR | 1.000 | | | | | |
| END | | | | | | |
| END | | | | | | |

## Table C-8. Partial SAPLUME Output for Horizontal Plume

FOR HAZARD LEVEL 1, WINDSPEED 1.500 m/s AND CATEGORY 6

THE HAZARDOUS CLOUD EXTENDS FROM $6.327 \times 10^{1}$ TO $3.126 \times 10^{3}$ METERS DOWNWIND AND HAS AN AREA OF $1.129 \times 10^{5}$ $m^2$

CLOUD BOUNDARIES

| X[a] (m) | WIDTH (m) | X (m) | WIDTH (m) | X (m) | WIDTH (m) | X (m) | WIDTH (m) | X (m) | WIDTH (m) |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | $1.000 \times 10^{-1}$ | 0.000 | $1.162 \times 10^{-1}$ | 0.000 | $1.350 \times 10^{-1}$ | 0.000 | $1.568 \times 10^{-1}$ | 0.000 |
| $1.822 \times 10^{-1}$ | 0.000 | $2.117 \times 10^{-1}$ | 0.000 | $2.460 \times 10^{-1}$ | 0.000 | $2.858 \times 10^{-1}$ | 0.000 | $3.320 \times 10^{-1}$ | 0.000 |
| $3.857 \times 10^{-1}$ | 0.000 | $4.482 \times 10^{-1}$ | 0.000 | $5.207 \times 10^{-1}$ | 0.000 | $6.050 \times 10^{-1}$ | 0.000 | $7.029 \times 10^{-1}$ | 0.000 |
| $8.166 \times 10^{-1}$ | 0.000 | $9.488 \times 10^{-1}$ | 0.000 | 1.102 | 0.000 | 1.281 | 0.000 | 1.488 | 0.000 |
| 1.729 | 0.000 | 2.009 | 0.000 | 2.334 | 0.000 | 2.711 | 0.000 | 3.150 | 0.000 |
| 3.660 | 0.000 | 4.252 | 0.000 | 4.940 | 0.000 | 5.740 | 0.000 | 6.669 | 0.000 |
| 7.748 | 0.000 | 9.002 | 0.000 | $1.046 \times 10^{1}$ | 0.000 | $1.215 \times 10^{1}$ | 0.000 | $1.412 \times 10^{1}$ | 0.000 |
| $1.640 \times 10^{1}$ | 0.000 | $1.906 \times 10^{1}$ | 0.000 | $2.214 \times 10^{1}$ | 0.000 | $2.572 \times 10^{1}$ | 0.000 | $2.989 \times 10^{1}$ | 0.000 |
| $3.472 \times 10^{1}$ | 0.000 | $4.034 \times 10^{1}$ | 0.000 | $4.687 \times 10^{1}$ | 0.000 | $5.446 \times 10^{1}$ | 0.000 | $6.327 \times 10^{1}$ | 5.555 |
| $7.351 \times 10^{1}$ | 2.627 | $8.540 \times 10^{1}$ | 2.739 | $9.922 \times 10^{1}$ | 2.955 | $1.153 \times 10^{2}$ | 3.294 | $1.339 \times 10^{2}$ | 3.768 |
| $1.556 \times 10^{2}$ | 4.385 | $1.808 \times 10^{2}$ | 5.149 | $2.101 \times 10^{2}$ | 6.066 | $2.441 \times 10^{2}$ | 7.143 | $2.835 \times 10^{2}$ | 8.388 |
| $3.294 \times 10^{2}$ | 9.811 | $3.827 \times 10^{2}$ | $1.142 \times 10^{1}$ | $4.447 \times 10^{2}$ | $1.324 \times 10^{1}$ | $5.167 \times 10^{2}$ | $1.527 \times 10^{1}$ | $6.003 \times 10^{2}$ | $1.701 \times 10^{1}$ |
| $6.974 \times 10^{2}$ | $1.856 \times 10^{1}$ | $8.103 \times 10^{2}$ | $2.005 \times 10^{1}$ | $9.414 \times 10^{2}$ | $2.140 \times 10^{1}$ | $1.094 \times 10^{3}$ | $2.257 \times 10^{1}$ | $1.271 \times 10^{3}$ | $2.345 \times 10^{1}$ |
| $1.476 \times 10^{3}$ | $2.393 \times 10^{1}$ | $1.715 \times 10^{3}$ | $2.384 \times 10^{1}$ | $1.993 \times 10^{3}$ | $2.295 \times 10^{1}$ | $2.315 \times 10^{3}$ | $2.082 \times 10^{1}$ | $2.690 \times 10^{3}$ | $1.653 \times 10^{1}$ |
| $3.126 \times 10^{3}$ | 5.139 | | | | | | | | |

[a]x = Downwind distance.

extends from about 60 m to about 12 km downwind, covering an area of approximately $10^6$ $m^2$.

The above results are close to those predicted by SLAB. The higher result is about 50% larger than that predicted by SLAB. However, the difference is within the range of uncertainties expected for these dispersion models. As noted above, the neglect of dry deposition means that the predictions are likely to be conservative.

## REFERENCES


AIChE, 1989. *Guidelines for Chemical Process Quantitative Risk Analysis*. American Institute of Chemical Engineers, NY.

Alp, E., et al., 1990. *An Approach for Estimating Risk to Public Safety from Uncontrolled Sour Gas Releases*. ERCB Report 90-B (10 Volumes), Prepared by Concord Environmental Corporation for Energy Resources Conservation Board, Calgary, Alberta.

Briggs, G.A., 1973. *Lift-Off of Buoyant Gas Initially on the Ground*. National Oceanic and Atmospheric Administration, Atmospheric Turbulence and Diffusion Laboratory File ATDL 83, Oak Ridge, TN.

Ermak, D.L., 1989. *User's Manual for the SLAB Model, An Atmospheric Dispersion Model for Denser-than-Air Releases*, Lawrence Livermore National Laboratory.

Layton, D.W., et al. 1983. *Accidental Releases of Sour Gas From Wells and Collection Pipelines in the Overthrust Belt: Calculating and Assessing Potential Health and Environmental Risks*. Lawrence Livermore National Laboratory Report UCRL-53411, Prepared for the Division of Fluid Mineral Operations, Bureau of Land Management, U.S. Department of the Interior, Washington, DC.

Quest, 1992. *Hazards Analysis/Risk Analysis Study of Union Pacific Resources Company's Wahsatch Gas Gathering Pipeline System*. Prepared by Quest Consultants, Inc., for Union Pacific Resources Company, Fort Worth, TX.

(Please read Instructions on the reverse before completing)

| | | |
|---|---|---|
| 1. REPORT NO. EPA-453/R-93-045 | 2. | 3. RECIPIENT'S ACCESSION NO. |
| 4. TITLE AND SUBTITLE Report to Congress on Hydrogen Sulfide Air Emissions Associated with the Extraction of Oil and Natural Gas | | 5. REPORT DATE October 1993 |
| | | 6. PERFORMING ORGANIZATION CODE |
| 7. AUTHOR(S) OAQPS - project lead - Laurel Driver OSWER - project lead - Ed Freedman | | 8. PERFORMING ORGANIZATION REPORT NO. |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS | | 10. PROGRAM ELEMENT NO. |
| | | 11. CONTRACT/GRANT NO. 68-D2-0065 |
| 12. SPONSORING AGENCY NAME AND ADDRESS Office of Air Quality Planning and Standards (MD-13) U.S. Environmental Protection Agency Research Triangle Park, NC 27711 | | 13. TYPE OF REPORT AND PERIOD COVERED Final |
| | | 14. SPONSORING AGENCY CODE |

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Under section 112(n)(5) of the Clean Air Act Amendments of 1990, the EPA is required to submit this report which assesses the hazards to public health and the environment resulting from the emission of hydrogen sulfide associated with the extraction of oil and natual gas. This assessment is designed to build upon work done under section 8002(m) of the Solid Waste Disposal Act and to reflect consultation with the States. The report includes a review of existing State and industry control standards, techniques, and enforcement and includes recommendations for control of hydrogen sulfide emissions from these sources.

The Office of Air Quality Planning and Standards and the Office of Solid Waste and Emergency Response completed this report on a joint effort. This report provides the information currently available on each of the elements listed above.

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| Hydrogen Sulfide<br>Air Emission Sources<br>Hydrogen Sulfide Report to Congress<br>Clean Air Act Amendment of 1990<br>Oil and Natural Gas Extraction | | |

| | | |
|---|---|---|
| 18. DISTRIBUTION STATEMENT Unlimited | 19. SECURITY CLASS (This Report) unclassified | 21. NO. OF PAGES |
| | 20. SECURITY CLASS (This page) unclassified | 22. PRICE |

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

# Hydrogen Sulfide, Oil and Gas, and People's Health

**By**

Lana Skrtic

Submitted in partial satisfaction of the requirements for the degree of

**Master's of Science**

**May 2006**

**Energy and Resources Group**
**University of California, Berkeley**

*Table of Contents*


1. Introduction ..... 3
2. Hydrogen Sulfide in the Environment ..... 4
3. Hydrogen Sulfide and Oil and Gas ..... 5
4. Hydrogen Sulfide Emissions from Oil and Gas Facilities ..... 7
5. Human Health Effects from Exposure to Hydrogen Sulfide ..... 10
    5.1 Literature Review - Acute Exposure ..... 14
    5.2 Literature Review - Chronic Exposure ..... 19
6. Regulations and Recommendations for Exposure to Hydrogen Sulfide ..... 27
    6.1 Federal Recommendations and Regulations ..... 27
    6.2 State Regulations ..... 33
        6.2.1 Special $H_2S$ Monitoring Studies ..... 35
            6.2.1.1 Arkansas ..... 35
            6.2.1.2 Colorado ..... 36
            6.2.1.3 Louisiana ..... 37
            6.2.1.4 New Mexico ..... 37
            6.2.1.5 North Dakota ..... 39
        6.2.2 Routine Monitoring ..... 40
            6.2.2.1 California ..... 40
            6.2.2.2 Oklahoma ..... 42
            6.2.2.3 Texas ..... 44
7. Evidence From People Living Near Oil and Gas Operations ..... 44
8. Concluding Remarks ..... 51
Bibliography ..... 55
Appendix A: Guidelines for Occupational Exposure to Hydrogen Sulfide ..... 58
Appendix B: State Ambient Hydrogen Sulfide Standards ..... 60
Appendix C: California Air Districts ..... 62
Appendix D: Interview Narratives ..... 63


**Tables**


1. Health Effects Associated with Hydrogen Sulfide ..... 13
2. Summary of Studies ..... 26
3. Summary of Monitoring Data from New Mexico Study ..... 38
4. California $H_2S$ Monitoring Sites ..... 41
5. Summary of Interviews ..... 43


**Figures**


1. Map of Major $H_2S$-prone Areas in the Continental United States ..... 7
2. State Ambient $H_2S$ Regulations ..... 33
3. Tulsa $H_2S$ Monitoring Data ..... 44

## 1. Introduction

This paper documents impacts on human health caused by exposure to hydrogen sulfide ($H_2S$) associated with oil and natural gas development. I begin with a brief background on hydrogen sulfide, its presence in oil and natural gas, and possible emission sources from various oil and gas operations. I then present a review of literature[1] from available public health, epidemiology, and industrial health publications, as well as of sources from regulatory and environmental agencies, that addresses human health impacts from exposure to $H_2S$. The Literature Review section first covers studies of health effects from acute exposure to relatively high concentrations of $H_2S$. I then review the literature documenting human health effects from chronic exposure to lower ambient $H_2S$ levels. Both kinds of exposure – acute and chronic – can be expected to occur near oil and gas operations. From the available sources, I construct a table of human health effects associated with different levels of hydrogen sulfide and different lengths of exposure. Reviewing studies on the effects of $H_2S$ exposure on laboratory animals is beyond the scope of this study.

Next, I present current federal and state regulations and recommendations pertaining to exposure to hydrogen sulfide. Many recommendations established to protect human health are based on crude exposure estimates or on extrapolation from animal studies. The federal government does not regulate ambient $H_2S$ levels, but many states do. Three states conduct routine monitoring of ambient $H_2S$ levels, and several others have monitored $H_2S$ as part of specific projects. I present the available monitoring data, as well as anecdotal evidence about $H_2S$ emissions and human health concerns that I obtained from conversations with staff at state environmental agencies.

[1] I searched on-line catalogs including Web of Science and Environmental Sciences and Pollution Management, and tracked down relevant references listed within each article.

The final component of my research consists of informal interviews with people living near oil and gas operations who have been, or believe they have been, exposed to hydrogen sulfide and believe they are experiencing adverse heath effects due to exposure. Enough evidence emerges from literature searches and reviews, environmental health professionals, available monitoring data, and personal stories to warrant more research. Although the evidence is patchy, the potential for health risks is real and the stakes are high. More monitoring and regulation are required to adequately protect human health.

## 2. Hydrogen Sulfide in the Environment

Approximately 90 percent of the sources that emit hydrogen sulfide into the air are natural.[2] Hydrogen sulfide is released into the air as a product of the decomposition of dead plant and animal material,[3] especially when this occurs in wet conditions with limited oxygen, such as in swamps. Hot springs, volcanoes, and other geothermal sources also emit $H_2S$.

Anthropogenic releases of $H_2S$ into the air result from industrial processes, primarily from the extraction and refining of oil and natural gas and from paper and pulp manufacturing,[4] but the gas is also present at sewage treatment plants, manure-handling plants, tanneries, and coke oven plants.[5]

---

[2] EPA, "Report to Congress on Hydrogen Sulfide Air Emissions Associated with the Extraction of Oil and Natural Gas." EPA-453/R-93-045, October 1993. " p.III-4.
[3] Decomposition of dead organic matter (DOM) by fungi, actinomycetes, and bacteria releases hydrogen sulfide from sulfur-containing proteins and from the direct reduction of sulfate (SO4+).
[4] New York State Department of Health: available at http://www.health.state.ny.us/nysdoh/environ/btsa/sulfide.htm
[5] "Public Health Statement for Hydrogen Sulfide," Agency for Toxic Substances and Disease, September 2004. Available at http://www.atsdr.cdc.gov/toxprofiles/tp114-c1.pdf

## 3. Hydrogen Sulfide and Oil and Gas

Hydrogen sulfide is a naturally occurring component of crude oil and natural gas. Petroleum oil and natural gas are the products of thermal conversion of decayed organic matter (called kerogen) that is trapped in sedimentary rocks. High-sulfur kerogens release hydrogen sulfide during decomposition, and this $H_2S$ stays trapped in the oil and gas deposits.[6]

Methane ($CH_4$) is the predominant component of natural gas, comprising 70 to 90 percent, while other gaseous hydrocarbons, butane ($C_4H_{10}$), propane ($C_3H_8$), and ethane ($C_2H_6$), account for up to 20 percent. Contaminants present in natural gas, which have to be removed at natural gas processing facilities, include water vapor, sand, oxygen, carbon dioxide, nitrogen, rare gases such as helium and neon, and hydrogen sulfide.[7] In fact, hydrogen sulfide is the predominant impurity in natural gas.[8] The Environmental Protection Agency (EPA) classifies natural gas as *sour* when $H_2S$ is present "in amounts greater than 5.7 milligrams per normal cubic meters ($mg/Nm^3$) (0.25 grains per 100 standard cubic feet)."[9]

Sour gas is routinely 'sweetened' at processing facilities called desulfurization plants. Ninety five percent of the gas sweetening process involves removing the $H_2S$ by absorption in an amine solution, while other methods include carbonate processes, solid bed absorbents, and physical absorption.[10]

---

[6] EPA "Report to Congress on Hydrogen Sulfide Emissions", p.II-1.
[7] *Oil and Gas at Your Door? A landowner's guide to oil and gas development*. OGAP 2005. p.I-2.
[8] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.II-i.
[9] Environmental Protection Agency, AP 42, Fifth Edition, Volume I, Chapter 5: The Petroleum Industry, available at http://www.epa.gov/ttn/chief/ap42/ch05/final/c05s03.pdf
[10] EPA, "Petroleum Industry." P.5.3-1. For details on these and other technologies for 'sweetening' sour gas, see "Crystasulf Process for Desulfurizing Ultra-deep Natural Gas Near the Wellhead," presented at *Natural Gas Technologies II Conference and Exhibition,* February 2004. Phoenix, AZ. Ref. No. T04135. pp.5-9.

Between 15 to 25 percent of natural gas in the U.S. may contain hydrogen sulfide,[11] while worldwide, the figure could be as high as 30 percent. The exact number of sour wells in the United States is not known, though natural gas deposits in Arkansas, southeastern New Mexico, western Texas, and north-central Wyoming have been identified as sour.[12] Hydrogen sulfide occurs naturally in the geologic formations in the Rockies, the Midcontinent, Permian Basin, and Michigan and Illinois Basins.[13] As more natural gas development occurs in these areas, it is likely that the number of sour wells will increase, because new drilling is increasingly focused on deep gas formations that tend to be sour.[14] Although exact statistics on sour wells are not available, the EPA concedes that "the potential for routine $H_2S$ emissions [at oil and gas wells] is significant."[15]

The most comprehensive source on the distribution of sour gas is a report prepared by consultants for the Gas Technology Institute, formerly Gas Research Institute, a research, development, and training organization that serves the natural gas industry.[16] This report states that "Regions with the largest percentage of proven reserves with at least 4 ppm hydrogen sulfide are Eastern Gulf of Mexico (89 percent), Overthrust (77 percent), and Permian Basin (46

---

[11] Dalrymple, D.A., Skinner, F.D. and Meserole, N.P. 1991. *Investigation of U.S. Natural Gas Reserve Demographics and Gas Treatment Processes.* Topical Report, GRI-91/0019, Section 3.0, pp. 3-1 to 3-13. Gas Research Institute. And Hugman, R.H., Springer, P.S. and Vidas, E.H. *Chemical Composition of Discovered and Undiscovered Natural Gas in the United States: 1993 update*. Topical Report, GRI-93/0456. p. 1-3. Gas Research Institute. As cited in McIntush, K.E., Dalrymple, D.A. and Rueter, C.O. 2001. "New process fills technology gap in removing H2S from gas," *World Oil*, July, 2001.

[12] EPA, "Report to Congress on Hydrogen Sulfide Emissions". p. I-3.

[13] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p. I-3.

[14] Quinlan, M., 1996. "Evaluation of selected emerging sulfur recovery technologies," *GRI Gas Tips*, 3(1):26-35. In McIntush, K.E., Dalrymple, D.A. and Rueter, C.O. 2001. "New process fills technology gap in removing H2S from gas," *World Oil*, July, 2001.

[15] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-35.

[16] Energy and Environmental Analysis, Inc. for Gas Research Institute, "Chemical Composition of Discovered and Undiscovered Natural Gas in the Lower-48 United States," GRI 90/0248. November 1990. (mailed to me by librarian for Gas Technology Institute).

percent)."[17] Figure 1 illustrates the major $H_2S$ prone areas in the United States and identifies the basins.

**Figure 1.** ***Map of Major $H_2S$-prone Areas in the Continental United States***[18]

## 4. Hydrogen Sulfide Emissions from Oil and Gas Facilities

There has been some investigation of hydrogen sulfide emissions associated with oil and gas development.[19] In the Literature Review section, I summarize several studies that researched $H_2S$ emissions near oil and gas facilities. Several states' environmental departments have monitored $H_2S$ concentrations near oil and gas operations. My conversations with personnel at these agencies confirm that there are $H_2S$ emissions associated with oil and gas activities. I present the evidence from the state studies and my conversations with staff in the State Regulations section. Finally, the interviews I conducted with people living near oil and gas

[17] Energy and Environmental Analysis, Inc. for Gas Research Institute. pp.2-3.
[18] Energy and Environmental Analysis, Inc. for Gas Research Institute. p.1-13 and p.A-5.
[19] For example, Environmental Protection Agency, "Report to Congress on Hydrogen Sulfide Air Emissions Associated with the Extraction of Oil and Natural Gas." EPA-453/R-93-045, October 1993. and Tarver, Gary A. and Purnendu K. Dasgupta. "Oil Field Hydrogen Sulfide in Texas: Emission Estimates and Fate." *Environmental Science and Technology*. **31**: (12) 3669-3676. 1997.

sites attest to the presence of $H_2S$ in the ambient air. Detailed narratives of the interviews are in Appendix D.

Oil and gas operations may emit hydrogen sulfide, routinely or accidentally, during the extraction, storage, transport, or processing stage.[20] During of extraction, hydrogen sulfide may be released into the atmosphere at wellheads, pumps, piping, separation devices, oil storage tanks, water storage vessels, and during flaring operations.[21] Flares burn gases that cannot be sold as well as gases at points in the system where operating problems may occur, as a safety measure. Because it cannot be sold, hydrogen sulfide is routinely flared. Sulfur dioxide ($SO_2$) is the product of combusting hydrogen sulfide, but in the event of incomplete combustion, $H_2S$ may be emitted into the atmosphere.

Based on reviewing the available literature and the records of agencies to which accidental releases of hydrogen sulfide might be reported,[22] the EPA states that well blowouts, line releases, extinguished flares, collection of sour gas in low-lying areas, line leakage, and leakage from idle or abandoned wells are sources of documented accidental releases that have impacted the public, not just workers at of oil and gas extraction sites.[23] Well blowouts are uncontrolled releases from wells, and can occur during drilling, servicing, or production, as a result of a failed 'blowout preventer' during drilling or a failed subsurface safety valve during

---

[20] *Schlumberger Oilfield Glossary*, available at http://www.glossary.oilfield.slb.com/default.cfm

[21] EPA "Report on Hydrogen Sulfide Air Emissions," P.II-6. See Section II, pp.3 to 10 for details. A wellhead is the first piece of equipment where the oil leaves the ground. Pumps that extract the oil may leak at the seals. Piping connects the various machinery and storage units at an oil pad. Separation devices separate oil from gas and water, and pipes take the gas to a dehydrator, while other pipes direct water and oil to a heater-treater where the two are separated. The oil is then piped into an oil storage tank, and the water is piped into a produced water storage tank. Wellheads, pipes, and separation devices may leak hydrogen sulfide because of corrosion and embrittlement caused by the reaction of water with metal and $H_2S$, or due to poor maintenance and poor materials. The heater-treaters may release hydrogen sulfide due to high pressures or pressure changes above design specifications. Oil storage tanks may release hydrogen sulfide as a result of day-night temperature changes, volatilization, and filling operations. Produced water storage vessels may contain hydrogen sulfide dissolved in water that is brought up from the reservoir, or it may be produced by sulfate-reducing bacteria found in water and oil.

[22] State agencies, emergency response organizations, industry officials. EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-36.

[23] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-38.

production.[24] The release from a well blowout can last for an indefinite period.[25] After all economically recoverable oil and gas has been removed, the well needs to be plugged, or sealed. If a well is improperly sealed, hydrogen sulfide may routinely seep into the atmosphere. One study, discussed below, documented precisely this type of hydrogen sulfide emissions in Whaler's Cove, a community in Long Beach, California, where a townhouse development was built on a 1940s oil field. Additionally, hydrogen sulfide may be routinely or accidentally released into the atmosphere at oil refineries and natural gas processing facilities, including desulfurization plants.

Hydrogen sulfide emissions from oil and gas development may pose a significant human health risk, as the studies discussed below reveal. Workers in the oil and gas industry are trained to recognize and respond to high-concentration accidental releases of $H_2S$. The American Petroleum Institute (API), an oil and gas industry technical organization, publishes recommendations for practices that help prevent hazardous $H_2S$ concentrations from occurring in the workplace.[26] People living near oil and gas development sites may be chronically exposed to much lower, but nonetheless dangerous ambient $H_2S$ levels, as well as to accidental high-concentration releases. A 1993 EPA report on the emissions of hydrogen sulfide from oil and gas extraction acknowledges that because of the proximity of oil and gas wells to areas where people live, the affected population may be large.[27]

Additionally, the "Public Health Statement for Hydrogen Sulfide," a public health advisory summarizing the longer $H_2S$ Toxicological Profile issued by the Centers for Disease Control and Prevention's Agency for Toxic Substances and Disease Registry (ATSDR),

---

[24] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-45.
[25] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-49.
[26] API Recommended Practice (RP) 54, *Recommended Practice for Occupational Safety for Oil and Gas Well Drilling and Servicing Operations* and API RP 49, *Safe Drilling of Wells Containing Hydrogen Sulfide*.
[27] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-65.

acknowledges that "As a member of the general public, you might be exposed to higher-than-normal levels of hydrogen sulfide if you live near a waste water treatment plant, a gas and oil drilling operation, a farm with manure storage or livestock confinement facilities, or a landfill. Exposure from these sources is mainly from breathing air that contains hydrogen sulfide."[28] The ATSDR also reports that higher than normal ambient "levels [of hydrogen sulfide] (often exceeding 90 ppb) have been detected in communities living near natural sources of hydrogen sulfide or near industries releasing hydrogen sulfide."[29]

**5. Human Health Effects from Exposure to Hydrogen Sulfide**

Human health effects of exposure to hydrogen sulfide, an irritant and an asphyxiant, depend of the concentration of the gas and the length of exposure. Background ambient levels of $H_2S$ in urban areas range from 0.11 to 0.33 ppb, while in undeveloped areas concentrations can be as low as 0.02 to 0.07 ppb.[30] A rotten egg odor characterizes $H_2S$ at low concentrations, and some people can detect the gas by its odor at concentrations as low as 0.5 ppb.[31] About half of the population can smell $H_2S$ at concentrations as low as 8 ppb, and more than 90% can smell it at levels of 50 ppb.[32] Hydrogen sulfide, however, is odorless at concentrations above 150 ppb, because it quickly impairs the olfactory senses.[33] Prolonged exposure to concentrations below

---

[28] "Public Health Statement for Hydrogen Sulfide," Agency for Toxic Substances and Disease, September 2004. Available at http://www.atsdr.cdc.gov/toxprofiles/tp114-c1.pdf
[29] ATSRD, Ch2, p.1.
[30] Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Toxicological profile for hydrogen sulfide (*Draft for Public Comment*). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Chapter 2, p.1.
[31] New York State Department of Health: available at http://www.health.state.ny.us/nysdoh/environ/btsa/sulfide.htm
[32] Collins, P. and Lewis, L. 2000. *Hydrogen Sulfide: Evaluation of Current California Air Quality Standard with Respect to Protection of Children.* Prepared for California Air Resources Board and California Office of Environmental Health Hazard Assessment. In: Summary of the toxicity assessment of hydrogen sulfide conducted by the Secretary's Scientific Advisory Board on Toxic Air Pollutants. http://daq.state.nc.us/toxics/studies/H2S
[33] Knight, Laura D., MD, and S. Erin Presnell, MD. 2005. "Death by Sewer Gas: Case Report of a Double Fatality and Review of the Literature." *The American Journal of Forensic Medicine and Pathology*. p.183.

150 ppb can also cause olfactory fatigue.[34] This effect of disabling the sense of smell at levels that pose serious health risks and possibly are life-threatening is one especially insidious aspect of hydrogen sulfide exposure. Odor is not necessarily a reliable warning signal of the presence of $H_2S$.

Most effects to humans occur from inhalation, though exposure generally also affects the eyes. Because most organ systems are susceptible to its effects, hydrogen sulfide is considered a broad spectrum toxicant.[35] The organs and tissues with exposed mucous membranes (eyes, nose) and with high oxygen demand (lungs, brain) are the main targets of hydrogen sulfide.[36] Hydrogen sulfide acts similarly to hydrogen cyanide, interfering with cytochrome oxidase and with aerobic metabolism.[37] Essentially, hydrogen sulfide blocks cellular respiration, resulting in cellular anoxia, a state in which the cells do not receive oxygen and die. The human body detoxifies hydrogen sulfide by oxidizing it into sulfate or thiosulfate by hemoglobin-bound oxygen in the blood or by liver enzymes.[38] Lethal toxicity occurs when $H_2S$ is present in concentrations high enough to overwhelm the body's detoxification capacity.[39]

At levels up to 100 to 150 ppm, hydrogen sulfide is a tissue irritant, causing keratoconjunctivitis (combined inflammation of the cornea and conjunctiva), respiratory irritation with lacrimation (tears) and coughing.[40] Skin irritation is also a common symptom. Instantaneous loss of consciousness, rapid apnea (slowed or temporarily stopped breathing), and

---

[34] Glass, D.C. "A Review of the Health Effects of Hydrogen Sulphide Exposure." *Annals of Occupational Hygiene*. 34:(3) p.323.
[35] Legator, Marvin S., et al.. "Health Effects from Chronic Low-Level Exposure to Hydrogen Sulfide." *Archives of Environmental Health*. **56:** (2) 123-131. March/April 2001. p.124.
[36] Legator, Marvin S., et al.. p.124.
[37] Knight, Laura D., MD, and S. Erin Presnell, MD. 2005. "Death by Sewer Gas: Case Report of a Double Fatality and Review of the Literature." *The American Journal of Forensic Medicine and Pathology*. p.183.
[38] Knight, 2005. p.184.
[39] Knight, 2005. p.184.
[40] Knight, 2005. p.183.

death may result from acute exposure to levels above 1,000 ppm.[41] At these higher levels, hydrogen sulfide is an asphyxiant.

The non-lethal effects can be summarized as *neurological* – consisting of symptoms such as dizziness, vertigo, agitation, confusion, headache, somnolence, tremulousness, nausea, vomiting, convulsions, dilated pupils, and unconsciousness, and *pulmonary* – with symptoms including cough, chest tightness, dyspnea (shortness of breath), cyanosis (turning blue from lack of oxygen), hemoptysis (spitting or coughing up blood), pulmonary edema (fluid in the lungs), and apnea with secondary cardiac effects.[42]

Table 1 lists the health effects associated with $H_2S$ exposures of varying durations. The table reports health effects that toxicological and epidemiological studies have attributed to specific concentrations (or a range of concentrations) of hydrogen sulfide. Table 1 also includes health effects of exposure to known concentrations of $H_2S$ that were self-reported by participants in the studies discussed below.

[41] Knight, 2005. p.183.
[42] Snyder, Jack W., MD, PhD. et al.. "Occupational Fatality and Persistent Neurological Sequelae After Mass Exposure to Hydrogen Sulfide." *American Journal of Emergency Medicine*. **13**: (2) 199-203. 1995. p.201.

**Table 1: Health Effects Associated with Hydrogen Sulfide**[43]

| Concentration (ppm) | Length of exposure | Effect | Source |
|---|---|---|---|
| *0.0057* | *Community/chronic* | *Eye and nasal symptoms, coughs, headaches and/or migraines* | *Partti-Pellinen, p.316.* |
| 0.003 – 0.02 | Immediate | Detectable odor | EPA Report 1993, p.III-5 |
| *0.01* | *Community/chronic* | *Neurophysiological abnormalities* | *Legator, p.124.* |
| *0.1 – 1* | *Not reported (n.r.)* | *Abnormal balance with closed eyes, delayed verbal recall, impaired color discrimination, decreased grip strength* | *Kilburn, 1999, p.210.* |
| 0.2 | n.r. | Detectable odor | Fuller, p.940 |
| 0.250 – 0.300 | Prolonged | Nuisance due to odor from prolonged exposure | Milby, p.194 |
| *1 – 5* | *n.r.* | *Abnormal balance with open and closed eyes, delayed verbal recall, impaired color discrimination, decreased grip strength, abnormal simple and choice reaction time, abnormal digit symbol and trailmaking.* | *Kilburn, 1999, p.210* |
| *2 – 8* | *Community* | *Malaise, irritability, headaches, insomnia, nausea, throat irritation, shortness of breath, eye irritation, diarrhea, and weight loss* | *EPA Report 1993, p. III-32.* |
| 10 | 10 minutes | Eye irritation, chemical changes in blood and muscle tissue after 10 minutes | New York State Department of rt |
| > 30 | Prolonged | Fatigue, paralysis of olfaction from prolonged exposure | Snyder, p.200 |
| 50 | n.r. | Eye and respiratory irritation | Fuller, p.940 |
| 50 – 100 | Prolonged | Prolonged exposure leads to eye irritation; eye irritation (painful conjunctivitis, sensitivity to light, tearing, clouding of vision) and serious eye injury (permanent scarring of the cornea) | Milby p.194; EPA Report 1993, |
| 150 – 200 | n.r. | Olfactory nerve paralysis | EPA Report 1993, p.III-6 |
| 200 | n.r. | Respiratory and other mucous membrane irritation | Snyder, p.200 |
| 250 | n.r. | Damage to organs and nervous system; depression of cellular metabolism | EPA Report 1993, p.III-5 |
| 250 | Prolonged | Possible pulmonary edema from prolonged exposure | Milby p.193 |
| 320 – 530 | n.r. | Pulmonary edema with risk of death | Kilburn (1999), p.212 |
| 500 | 30 minutes | systemic symptoms after 30 minutes | Fuller, p.940 |
| 500 –1000 | Immediate | Stimulation of respiratory system, leading to hyperpnoea (rapid breathing); followed by apnea (cessation of breathing) | EPA Report 1993, p.III-5 |
| 750 | Immediate | Unconsciousness, death | Fuller, p.940 |
| 1000 | Immediate | Collapse, respiratory paralysis, followed by death | Fuller, p.940, EPA Report 1993 p. |
| 750 – 1000 | Immediate | Abrupt physical collapse, with possibility of recovery if exposure is terminated; if not terminated, fatal respiratory paralysis | Milby, p.192 |
| 1000 – 2000 | n.r. | Immediate collapse with paralysis of respiration | Kilburn (1999), p.212 |
| 5000 | Immediate | Death | Fuller, p.940 |

[43] Italics signify concentrations and health effects in studies that rely on self-reporting of symptoms, usually in questionnaires.

*5.1 Literature Review - Acute Exposure*

The following studies focused on short-term exposure to relatively high levels of hydrogen sulfide, the kind of scenario that can be expected from an accidental release. There are many documented instances and peer-reviewed studies of serious health effects and deaths from exposure to relatively high concentrations of hydrogen sulfide.

Fuller and Suruda (2000), who reviewed Occupational Safety and Health Administration (OSHA) investigation records from 1984 to 1994, reported 80 deaths in the United States from occupational exposure to hydrogen sulfide, out of a total 18559 occupational death during this period.[44] Twenty-two of the 80 deaths were in the oil and gas industry.[45] These deaths occurred as a result of workers' exposure to accidental releases of hydrogen sulfide in high concentrations. The authors concluded that portable $H_2S$ meters or alarms could have prevented these deaths.[46]

In their 1997 study, Hessel et al. submitted a questionnaire about health effects from hydrogen sulfide exposure to 175 oil and gas workers in Alberta, Canada, a known region of sour gas. Of the 175 workers, one third reported having been exposed to $H_2S$, and 14 workers (8%) experienced knockdown,[47] a term for the loss of consciousness due to inhaling high concentrations of hydrogen sulfide. The workers who had experienced knockdown exhibited the respiratory symptoms of shortness of breath, wheezing while hurrying or walking up hill, and random wheezing attacks.[48] The investigators found no

---

[44] Fuller, Douglas C., MD, MPH, and Anthony J. Suruda, MD, MPH. "Occupationally Related Hydrogen Sulfide Deaths in the United States From 1984 to 1994." *Journal of Occupational and Environmental Medicine*. **42**:(9) 939-942. September 2000. p.940.
[45] Fuller and Suruda, p.941.
[46] Fuller and Suruda, p.942.
[47] Hessel, Patrick A., PhD. et al.. "Lung Health in Relation to Hydrogen Sulfide Exposure in Oil and Gas Workers in Alberta, Canada." *American Journal of Industrial Medicine*. **31**:554-557. 1997., p.555
[48] Hessel, pp.555-556.

"measurable pulmonary health effects as a result of exposure to $H_2S$ that were intense enough to cause symptoms but not intense enough to cause unconsciousness."[49] In other words, the workers who reported initially experiencing symptoms from $H_2S$ exposure did not report exhibiting any lingering respiratory symptoms at the time of the study. However, other kinds of long term effects could exist; indeed, the study itself acknowledged that long term effects of acute short term exposure have not been studied enough, and finds this lack "noteworthy."[50]

Milby and Baselt (1999) relied on a review of literature about hydrogen sulfide poisoning, and state that "A phenomenon referred to as 'knockdown' has been reported in oil field workers and others to describe sudden, brief loss of consciousness followed by immediate full recovery after short-lived exposure to very high concentrations of hydrogen sulfide (e.g., 750-1000 ppm)."[51] However, other studies have contested this claim of full recovery following a knockdown.

Kaye Kilburn, a medical doctor and professor of medicine at the University of Southern California, has devoted a considerable part of his career to studying and reporting on the adverse health effects of hydrogen sulfide. Refuting Milby and Baselt's (1999) finding that full recovery followed unconsciousness, or 'knockdown,' Kilburn states, "In 1989, for the first time, sensitive testing showed that, although survivors who had been unconscious looked all right, brain functions were impaired. Similar impairments were measured in people exposed to amounts below 50 ppm that had not caused unconsciousness. Next, subtle impairments of brain function were measured from

---

[49] Hessel, p..556.
[50] Hessel, p.555.
[51] Milby, Thomas H. MD, and Randall C. Baselt, PhD. "Hydrogen Sulfide Poisoning: Clarification of Some Controversial Issues." *American Journal of Industrial Medicine*. **35**: 192-195. 1999. p.192.

exposures to concentrations of less than 5 ppm in air."[52] Kilburn reported examining one oil field worker, Stan, who had experienced 'knockdown' on the job after exposure to 1 percent hydrogen sulfide concentration (or 9,999 ppm as Stan's meter recorded it.) Three years after the incident, while appearing physically healthy, Kilburn's tests of Stan revealed significant brain damage (IQ lowered to 77, though the previous IQ is not reported), severely impaired balance and motor function, and inability to recall stories and visual designs.[53]

Another study by Kilburn (2003)[54] reported long term effects of hydrogen sulfide exposure. Kilburn performed physiologic and psychological measurements on nineteen exposed and 202 unexposed subjects.[55] Ten of the nineteen subjects were exposed at work, including four at oil and gas sites, while the other nine were exposed in their residences, which were near various sources of $H_2S$.[56] The concentrations to which the subjects were exposed are not known. Exposure times ranged from twenty minutes to nine years, and Kilburn examined the subjects from 1.7 to 22 years after their exposures.[57] The study methods consisted of a questionnaire and a series of neurophysiological and neuropsychological tests. The neurophysiological tests measured simple reaction time, visual two-choice reaction time, balance, color recognition, and hearing, and the neuropsychological tests measured immediate memory recall, mood, and vocabulary.[58] Tension, depression, anger, fatigue, and confusion were all significantly elevated in the

---

[52] Kaye H. Kilburn. "Killer Molecules in Natural Gas." Chapter 7 in *Endangered Brains: How Chemicals Threaten Our Future*. Birmingham, AL: Princeton Scientific Publishers Company, Inc. 2004. p.78.
[53] Kilburn, (2004) p.79.
[54] Kilburn, Kaye H. "Effects of Hydrogen Sulfide on Neurobehavioral Function." *Southern Medical Journal*. **96**: (7) 639-646. 2003.
[55] Kilburn, (2003), p.640.
[56] Kilburn, (2003), p.640, see Table 1, p.641.
[57] Kilburn, (2003), p.640.
[58] Kilburn, (2003), pp.640-641.

exposed subjects compared to the control group. In addition, respiratory symptoms were more prevalent among the exposed subjects.[59] Even subjects who did not experience unconsciousness at the time of their exposure exhibited permanent neurobehavioral damage.[60]

The studies mentioned thus far focused on occupational exposure. They document the dangerous properties of hydrogen sulfide, as well as highlight the fact that more research is needed on the long term effects of even short duration exposures. There have been some studies of non-occupational exposure to relatively high $H_2S$ levels. The proximity of oil refineries, gas treatment and processing plants, and oil and gas wells to residences constitutes a likely source of $H_2S$ emissions and potentially poses a risk to people in a non-occupational setting.

Kilburn has studied the health effects of a series of explosions at an oil refinery in Wilmington, California, which occurred in October 1992. The explosions released unknown amounts of hydrogen sulfide into the air, making people ill in Wilmington, Torrance, Carson, Long Beach, and South Los Angeles.[61] Some street monitors recorded $H_2S$ concentrations as high as 24 ppm, and since no one died, Kilburn concluded that concentrations probably did not exceed 200 ppm. Seven thousand people who had been exposed and sickened filed a consolidated lawsuit against the refinery, and a random sample were examined three and a half years after the explosion for court proceedings.[62]

---

[59] Kilburn, (2003), p.643.
[60] Kilburn, (2003), p.644.
[61] Kilburn, (2004) p.77.
[62] 400 people were selected to represent the 7000 filing suit, and 120 were selected at random to be examined by a general practitioner. Then, 68 of the 120 were examined using sensitive neurobehavioral tests. Kilburn, (2004) p.81.

Persistent symptoms included impaired balance, delayed recall memory, elevated depression and confusion scores, and abnormally slow reaction times.[63]

As background to their 1987 study, which focused on methods of improving the prediction and management of public health risks associated with the development of sour gas wells, Layton and Cederwall[64] summarized studies of two incidents during which people were exposed to hydrogen sulfide released from gas operations. One occurred in 1950 in Poza Rica, Mexico, where 320 people were hospitalized and 22 died as a result of a major hydrogen sulfide release from a gas purification plant.[65] The second incident, known as the Lodgepole blowout, was a sour gas blowout in Alberta, Canada, in 1982. In this case, the hydrogen sulfide releases lasted for 67 days, and the affected people reported headaches, eye irritation, and various respiratory and gastrointestinal symptoms.[66] In both instances, there were no reliable measurements of $H_2S$ concentrations. In Alberta, maximum reported hourly concentrations were 15 ppm, and concentrations 100 kilometers away from the source were below 100 ppb, but residents there filed over a thousand complaints.[67] This study concluded that the hazard zone for sublethal effects around sour gas wells encompasses from less than 400 meters up to 6500 meters, while lethal exposure to hydrogen sulfide could occur as far as 2000 meters from the source.[68] Among the proposed recommendations for improving public safety is "preemptive land ownership,"[69] an issue which I revisit in the Concluding Remarks section. This study also stressed that

[63] Kilburn, (2004) p.81.
[64] Layton, David W. and Richard T. Cederwall. 1987. "Predicting and Managing the Health Risks of Sour-Gas Wells." *Journal of the Air Pollution Control Association.* **37**: 1185-1190.
[65] Layton and Cederwall, 1987. pp.1185-1186.
[66] Layton and Cederwall, 1987. p 1186.
[67] Layton and Cederwall, 1987. p 1186.
[68] Layton and Cederwall, 1987. p 1188.
[69] Layton and Cederwall, 1987. p 1187.

sublethal effects of hydrogen sulfide are not well studied and that the dose-response relationship at lower levels is not well characterized.[70]

*5.2 Literature Review - Chronic Exposure*

Literature is also available on the human health impacts of chronic exposure to relatively low concentrations of hydrogen sulfide. Generally, chronic exposure to low-level concentrations of hydrogen sulfide is associated with neurological symptoms that include fatigue, loss of appetite, irritability, impaired memory, altered moods, headaches, and dizziness.[71] At persistent concentrations of 0.250 to 0.300 ppm (250 to 300 ppb), the rotten egg odor of $H_2S$ creates a nuisance to communities, and exposure to such concentrations has been documented to affect quality of life by causing headaches, nausea, and sleep disturbances.[72]

Schiffman et al. (1995) evaluated the effect of odors emanating from swine operations on mood.[73] Although the source of odors were swine operations rather than oil and gas sites, the study is relevant because hydrogen sulfide caused the persistent odors, much as is the case near oil refineries and natural gas processing plants. This study concluded that continuously smelling odors is associated with "significantly more tension, more depression, less vigor, more fatigue, and more confusion."[74]

---

[70] Layton and Cederwall, 1987. p 1185.
[71] McGavran, Pat. "Literature Review of the Health Effects Associated with the Inhalation of Hydrogen Sulfide." Idaho Department of Environmental Quality, Boise, Idaho. June 19, 2001. p.3.
[72] Milby, 1999, p.194.
[73] Schiffman, Susan S., Elizabeth A. Sattely, et al.. "The Effect of Environmental Odors Emanating From Commercial Swine Operations on the Mood of Nearby Residents." *Brain Research Bulletin*. 37:4 369-375. 1995
[74] Schiffman et al., p.371.

One frequently cited study, by Partti-Pellinen et al. (1996), examined the health effects of chronic, low-level exposure to sulfur compounds, including hydrogen sulfide, near a paper and pulp mill in Finland.[75] They found that the exposed people experienced eye and nasal symptoms, coughs, and headaches or migraines much more frequently than the people in the control group, while acute respiratory infections also occurred more frequently in the study group.[76] Once again, the study acknowledged the lack of data on long term effects of low-dose, chronic exposure, and concluded that, at the very least, the exposure and odor make "everyday life uncomfortable."[77]

Legator et al. (2001) investigated the effects of chronic, low levels of hydrogen sulfide by surveying two exposed communities, Odessa, Texas, and Puna, Hawaii, and comparing the health findings with several control communities.[78] Due to emissions from industrial wastewater, ambient concentrations of $H_2S$ in Odessa, Texas, registered at 335 to 503 ppb over 8 hours, 101 to 201 ppb over 24 hours, with an annual average of 7 to 27 ppb.[79] Puna, Hawaii, is situated in a volcanically active area.[80] There were no reliable measurements of $H_2S$ levels at Puna—they ranged from less than 1 ppb to periodic highs of 200 to 500 ppb. The study relied on a multi-symptom health survey and found various adverse health effects associated with hydrogen sulfide exposure in the study populations. The health symptoms included central nervous system impacts (fatigue, restlessness,

---

[75] Partti-Pellinen, Kirsi, Marttila Olli, Vilkka Vesa, et al.. "The South Karelia Air Pollution Study: effects of low-level exposure to malodorous sulfur compounds on symptoms." *Archives of Environmental Health.* **51**. (4) 315-320 1996. The study looked at the main components of total reduced sulfur (TRS) compounds—hydrogen sulfide $H_2S$, methyl mercaptan, $CH_3SH$, dimethyl sulfide $[CH_3]_2S$, and dimethyl disulfide $[CH_3]_2S_2$.

[76] Partti-Pellinen et al.. Acute respiratory infections occurred 1.6 times per year in the study group as compared to 1.1 times per year in the control group.

[77] Partti-Pellinen, et al., p.320.

[78] Legator, Marvin S., et al.. "Health Effects from Chronic Low-Level Exposure to Hydrogen Sulfide." *Archives of Environmental Health*. **56:** (2) 123-131. March/April 2001.

[79] Legator, p.124.

[80] Since 1976, Puna is a site of geothermal energy production, and supplies about 30% of Hawaii's electricity. US Department of Energy.

depression, short term memory loss, balance, sleep problems, anxiety, lethargy, headaches, dizziness, tremors), respiratory system impacts (wheezing, shortness of breath, coughing), and various ear, nose, and throat symptoms.[81] This study also concluded with a call for more research:

> The findings in our study, taken together with previously reported data concerning adverse responses to $H_2S$, strongly mandate the need for continued research on the possible detrimental effects of chronic exposure to the toxic agent. This is of decided public health significance, given the relatively large segment of the population that is regularly exposed to low levels of $H_2S$.[82]

Kilburn has also studied health impacts from chronic exposure to lower concentrations of hydrogen sulfide. He examined a preacher and eighteen congregation members in Odessa, Texas, who lived downwind from an oil refinery and often smelled the characteristic rotten egg odor of $H_2S$, occasionally experiencing nausea and vomiting.[83] Kilburn observed impaired balance, delayed verbal recall for stories, and difficulty distinguishing colors among the people he studied in Odessa.[84] Workers and people living downwind of another oil refinery, in Nipoma Mesa near San Luis Obispo, California, also exhibited impaired reaction time, impaired balance, depression, and impaired recall memory.[85]

As a result of poorly plugged wells of an abandoned oil and gas field in Long Beach, California, people living in a community built on this location were exposed to hydrogen sulfide that collected under concrete foundations and crawl spaces of homes,

---

[81] Legator, pp.126-129.
[82] Legator, p.130.
[83] Kilburn, (2004) p.79.
[84] Kilburn, (2004) p.80.
[85] Kilburn, (2004) p.80.

and in a low lying area around a communal swimming pool.[86] The $H_2S$ measurements ranged from 0.1 ppm to 1 ppm, with several peaks up to 5 ppm.[87] Kilburn examined 24 people from this community, and recorded abnormal balance with closed eyes, delayed verbal recall, and impaired color discrimination and grip strength, as compared to a control group.[88]

As reported by the EPA,[89] two notable occasions of increased ambient concentrations of hydrogen sulfide occurred in Great Kanawha River Valley, West Virginia, in 1950, and in Terre Haute, Indiana, in 1964. In Terre Haute, ambient $H_2S$ concentrations ranged from 2 to 8 ppm, emanating from a lagoon. In West Virginia, the highest concentration was 293 ppb, but there is no information on other levels. In both cases, symptoms included malaise, irritability, headaches, insomnia, and nausea, while the people exposed in Terre Haute also reported, among other effects, throat irritation, shortness of breath, eye irritation, diarrhea, and weight loss.[90] These incidents provide some evidence of health impacts from chronic exposure to ambient levels of hydrogen sulfide in the range that may be expected to occur near oil and gas sites.

Tarver and Dasgupta (1997) measured hydrogen sulfide concentrations near several oil fields in western Texas.[91] Although the researchers were studying the effects of increased anthropogenic sources of sulfur emissions on the sulfur cycle, the authors nevertheless gathered data that is pertinent to my research. The study found nighttime

---

[86] Kilburn, Kaye H. "Evaluating health effects from exposure to hydrogen sulfide: central nervous system dysfunction." *Environmental Epidemiology and Toxicology*. **1**:207-216. 1999. p.208.
[87] Kilburn (1999), p.208.
[88] Kilburn, (1999), p.210.
[89] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-32. For the entire paragraph.
[90] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-32
[91] Tarver, Gary A. and Purnendu K. Dasgupta. "Oil Field Hydrogen Sulfide in Texas: Emission Estimates and Fate." *Environmental Science and Technology*. **31**: (12) 3669-3676. 1997.

maximum $H_2S$ concentrations between 1 and 5 ppb.[92] While this concentration of hydrogen sulfide is only enough to produce an odor, a persistent odor can be a nuisance, and has been associated with increased tension, depression, fatigue, confusion, and decreased vigor.[93]

Some evidence exists on the effects of hydrogen sulfide on the reproductive system. Xu et al. (1998) conducted a retrospective epidemiological study to assess the association between spontaneous abortion and exposure to petrochemicals.[94] By reviewing the plant employment records, which also contain medical information, the researchers identified over 3000 women from the Beijing Yanshan Petrochemical Corporation who had been pregnant. Trained interviewers administered a questionnaire to gather information on the subjects' reproductive history, pregnancy outcomes, employment history, occupational exposure, smoking habits, alcohol consumption, indoor air pollution, diet, and demographic variables.[95] The study found that "exposure to petrochemicals, specifically benzene, gasoline, and hydrogen sulphide is significantly associated with increased frequency of spontaneous abortion."[96] Each chemical was individually found to have a statistically significant effect on the frequency of spontaneous abortion. Although the exposures mainly occurred in maintenance operations or due to accidental leaks and spillages,[97] rather than being chronic low level exposures,[98] this study is nevertheless important for the link it established between hydrogen sulfide

---

[92] Tarver and Dasgupta, p.3673.
[93] Schiffman et al. Discussed above on p.18.
[94] Xu, Xiping, Sung-Il Cho, et al.. "Association of petrochemical exposure with spontaneous abortion." *Occupational and Environmental Medicine*. 55: 31-36. 1998.
[95] Xu et al., p.31.
[96] Xu et al., p.34.
[97] Xu et al., p.35.
[98] The study acknowledged that "at lower exposures, the reproductive effects of hydrogen sulphide have not been determined, although it has been shown to enhance the fetal toxicity of carbon disulphide." Xu et al., pp.34-35.

and effects on the reproductive system. According to one personal account recounted below, hydrogen sulfide exposure is associated with spontaneous abortions in cattle as well as other reproductive effects in animals.

Most studies acknowledge that there is a need for more research on the health impacts of chronic exposure to lower concentrations of $H_2S$. Although the health effects are not well documented,[99] many studies recognize the potential for harm. In 1993, the EPA prepared an in-depth report on hydrogen sulfide emissions associated with oil and gas extraction.[100] The report matched available routine emissions data from oil and gas sites with studies documenting health effects of these levels, and assessed the risk of accidental releases, to determine whether these warrant a national control strategy.[101] Although the report acknowledged that oil refineries and gas processing plants are a major possible source of $H_2S$, these were not included in the analysis because they fall outside the definition of the term 'extraction.'[102] The report also excluded exploration and well development activities. Each of these areas of oil and gas operations is a potential source of hydrogen sulfide emissions.

The report concluded that "the potential for human and environmental exposures from routine emissions of $H_2S$ from oil and gas wells exists, but insufficient evidence exists to suggest that these exposures present any significant threat,"[103] and that "there appears to be no evidence that a significant threat to public health or the environment exists from routine $H_2S$ emissions from oil and gas extraction."[104] The EPA reached this

[99] New York State Department of Health, http://www.health.state.ny.us/nysdoh/environ/btsa/sulfide.htm
[100] EPA, "Report to Congress on Hydrogen Sulfide Emissions."
[101] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-1.
[102] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.I-4.
[103] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.iii.
[104] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.V-1.

conclusion "from the limited data available."[105] However, because, as the report itself acknowledged, there is not enough information on ambient air quality around well sites,[106] the conclusion that there are no health risks is ill founded. A call for further research would have been more appropriate, but strikingly, the "Research and Further Studies" section of the last chapter does not recommend additional research of routine hydrogen sulfide emissions and health effects.

[105] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.V-1.
[106] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-1.

**Table 2: Summary of Studies**

| | Author(s) / Date | Discipline | Motivation for Study / Summary of Findings |
|---|---|---|---|
| Acute Exposure | Fuller and Suruda (2000) | Medicine | To determine the number of occupational deaths related to hydrogen sulfide; review of OSHA records; acute exposure |
| | Milby and Baselt (1999) | Medicine / Toxicology | Review of literature on hydrogen sulfide; focusing on neurotoxic effects of acute exposure, effects on the lungs, diagnosis of poisoning, and community exposure issues. |
| | Hessel et al. (1997) | Public Health | To assess pulmonary health effects of oil and gas workers in Alberta, Canada; administered questionnaire to 175 workers |
| | Snyder et al. (1995) | Medicine | To assess neurological problems from exposure to hydrogen sulfide; review of case reports from an incident of mass exposure to H2S in New Jersey; calls for annual neurological and neuropsychological testing of exposed subjects to enhance knowledge of long term effects |
| Chronic Exposure | Parti-Pellinen et al. (1996) | Medicine / Public Health | Examined health effects chronic, low-level exposure to sulfur compounds, including H2S, near a paper and pulp mill; administered cross-sectional questionnaire to 336 subjects and to a reference community; increased frequency of eye and nasal symptoms, coughs, and headaches or migraines, and acute respiratory infections. |
| | Legator et al. (2001) | Medicine / Toxicology / Public Health | Investigate effects of chronic exposure to low levels of hydrogen sulfide; multi-symptom health survey submitted to two exposed communities – Odessa, Texas and Puna, Hawaii, and to control communities; found central nervous system impacts: fatigue, restlessness, depression, short term memory loss, balance and sleep problems, anxiety, lethargy, headaches, dizziness, tremors; respiratory system impacts: wheezing, shortness of breath, coughing; and various ear, nose, and throat symptoms. |
| | Tarver and Dasgupta (1997) | Chemistry | To determine hydrogen sulfide concentrations near oil fields in Western Texas |
| | Xu et al. (1998) | Medicine / Epidemiology | To determine effects of exposure to hydrogen sulfide on the reproductive system; conducted a retrospective epidemiological study to assess the association between spontaneous abortion and exposure to petrochemicals in Beijing, China; found an association. |
| | Kilburn (1999) | Epidemiology | To determine long-term effects of exposure to hydrogen sulfide; examined and submitted a questionnaire to four groups of people that were exposed to hydrogen sulfide (from boreholes in the ground, downwind of a refinery, due to an oil refinery explosion, and a group of people exposed to odors); found abnormal balance, delayed verbal recall, impaired color discrimination and grip strength. |
| | Schiffman et al. (1995) | Psychiatry | To determine the effect of persistent environmental odors on the mood of people living near the source of odors; submitted a questionnaire to 44 subjects and 44 controls; found more tension, depression, fatigue, and confusion, and less vigor among the exposed subjects. |
| | Kilburn (2003) | Epidemiology | To measure long term effects of hydrogen sulfide exposure – various lengths of exposure and various concentrations; submitted a questionnaire, and performed neuropsychological and neurophysiological tests on 19 exposed subjects and 202 unexposed subjects; found elevated tensions, depression, anger, fatigue, and confusion, and more prevalent respiratory symptoms among exposed subjects. |
| Other | Layton and Cederwall (1987) | Engineering / Public Health | Methods for improving the prediction and management of public health risks associated with development of sour gas wells |
| | Knight and Presnell (2005) | Medicine / pathology | Review of literature on H2S toxicology; case study of two fatalities due to occupational exposure to H2S |

## 6. Regulations and Recommendations for Exposure to Hydrogen Sulfide

### *6.1 Federal Recommendations and Regulations*

At the federal level, some regulations and recommendations exist to protect humans from the health effects of exposure to hydrogen sulfide. Regulations are laws that can be enforced by agencies such as the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Occupational Safety and Health Administration (OSHA). Recommendations, on the other hand, do not carry the force of law, and are determined by agencies such as the National Institute for Occupational Safety and Health (NIOSH) and the Agency for Toxic Substances and Disease Registry (ATSDR), both part of the federal Centers for Disease Control and Prevention (CDC).

The American Conference of Governmental Industrial Hygienists (ACGIH), a longstanding member-based organization committed to promoting worker health and safety, also recommends exposure limits for various substances. The current ACGIH hydrogen sulfide standards are 10 ppm for the Threshold-Limit Value-Time Weighted Average (TLV-TWA), and 15 ppm for the TLV short term exposure limit (TLV-STEL). The TVL-TWA is the time-weighted average concentration to which workers can be routinely and consistently exposed over an 8-hour workday and 40-hour workweek without adverse effect. The TVL-STEL is the concentration to which workers can be exposed for short periods of time without suffering adverse health effects. The ACGIH updates its standards annually, and can relatively quickly modify its standards in response to new research.[107]

[107] EPA "Report to Congress on Hydrogen Sulfide Emissions," p.III-10.

OSHA began setting standards for workers' exposure to hazardous substances in the 1970s, and initially adopted the ACGIH values.[108] The current OSHA workplace standard for $H_2S$ exposure is 10 parts per million (ppm), while the exposure times are longer than the ACGIH recommends. In more detail, according to OSHA, "Exposures shall not exceed 20 ppm (ceiling) with the following exception: if no other measurable exposure occurs during the 8-hour work shift, exposures may exceed 20 ppm, but not more than 50 ppm (peak), for a single time period up to 10 minutes."[109] The OSHA regulations do not specify an 8-hour time weighted average (TWA) for $H_2S$. Exposure to these concentrations even for the seemingly short duration of 10 minutes can nevertheless result in eye and respiratory irritation, according to several sources. The NIOSH recommended exposure limit to the OSHA 10 ppm standard is 10 minutes, and its Immediately Dangerous to Life or Health (IDLH) $H_2S$ concentration is 100 ppm.[110] OSHA standards have the force of law, while ACGIH's and NIOSH's levels are only recommendations.

It is important to note that OSHA standards apply only to workplaces and not to domestic situations or residences. The human data on which the standards are based are from uncontrolled exposure incidents, so the levels of exposure are crudely estimated.[111] In general, the controlled exposure data is derived from animal studies and then extrapolated to humans. As one study discussed above summed up, "a precise ratio with which to predict human effects on the basis of the ratio of rat-to-human effects is

---

[108] EPA "Report to Congress on Hydrogen Sulfide Emissions," p.III-10.
[109] Occupational Safety and Health Administration, 29 CFR 1910.1000, available at http://www.osha.gov/dts/chemicalsampling/data/CH_246800.html
[110] NIOSH is a department within the Centers for Disease Control and Prevention. See http://www.cdc.gov/niosh/npg/npgd0337.html for NIOSH's $H_2S$ exposure recommendations.
[111] Guidotti, Tee L. 1994. "Occupational exposure to hydrogen sulfide in the sour gas industry: some unresolved issues." *International Archives of Occupational and Environmental Health*. p.157.

lacking."[112] Further, the standards are based on the expected effects of hydrogen sulfide on healthy adult males, so people who are young, old, or have compromised immune systems may be at risk at considerably lower concentrations of $H_2S$. Additionally, exposure to hydrogen sulfide may affect the human reproductive system, as determined in the study by Xu et al. and reported above, so standards based on males may not protect women's reproductive health.

In addition to general standards for workplace inhalation exposure, OSHA specifically sets standards for industries in which hydrogen sulfide occurs in quantities exceeding 1500 pounds, in their Process Safety Management of Highly Hazardous Chemicals Standard (1910-119). Significantly, the oil and gas industry is exempt from this standard.[113] According to the 1993 EPA report, the reason OSHA gave for this exemption is that OSHA "continues to believe that oil and gas well drilling and servicing operations should be covered in a standard designed to address the uniqueness of the industry."[114] OSHA also proposed a monitoring program for hydrogen sulfide for drilling and service operations that occur in areas where $H_2S$ exposure is a potential risk.[115] Neither of these exists at the time of writing.

The 1990 Clean Air Act is the primary federal law that regulates air pollution. The EPA sets the levels of various air pollutants, including the National Ambient Air Quality Standards (NAAQS) for six criteria pollutants and the National Emissions Standards for Hazardous Air Pollutants (NESHAPs) for another 188 substances commonly referred to as

---

[112] Kilburn, Kaye H. "Effects of Hydrogen Sulfide on Neurobehavioral Function." 2003. p.639.
[113] OSHA, http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9760
[114] As quoted in EPA "Report to Congress on Hydrogen Sulfide Emissions," p.IV-28.
[115] EPA "Report to Congress on Hydrogen Sulfide Emissions," p.IV-30.

HAPs.[116] The EPA does not regulate hydrogen sulfide as one of its criteria pollutants nor as one of the HAPs under the 1990 Clean Air Act. When George Bush, Sr. signed the Clean Air Act in 1990, $H_2S$ was not among the 188 chemicals on the final HAPs list to be regulated, despite the calls of public interest groups and government scientists, some even within the EPA, for its inclusion. Hydrogen sulfide had been on the proposed original list of hazardous substances,[117] and was removed from this list as a result of successful efforts by the oil and gas, chemical, and paper industries.[118] For instance, the American Petroleum Institute, representing the interests of the oil and gas industry, argued that $H_2S$ emissions are an "accidental-release issue" rather than a routine one,[119] and that $H_2S$ therefore should not be regulated as one of the Clean Air Act's Hazardous Air Pollutants. This lack of an EPA standard has prompted one newspaper to label hydrogen sulfide "the least regulated common poison."[120]

Hydrogen sulfide is on the EPA's list of Extremely Hazardous Substances,[121] another category under the Clean Air Act, which regulates substances "known or may be anticipated to cause death, injury, or serious adverse effects to human health or the

---

[116] According to the EPA, "Hazardous air pollutants, also known as toxic air pollutants or air toxics, are those pollutants that cause or may cause cancer or other serious health effects, such as reproductive effects or birth defects, or adverse environmental and ecological effects." http://www.epa.gov/ttn/atw/pollsour.html

[116] Interestingly, hydrogen sulfide remained on the list as a result of "administrative error" until it was removed by a Senate Joint Resolution on August 1, 1991. See http://www.epa.gov/ttn/atw/pollutants/atwsmod.html for details.

[117] Interestingly, hydrogen sulfide remained on the list as a result of "administrative error" until it was removed by a Senate Joint Resolution on August 1, 1991. See http://www.epa.gov/ttn/atw/pollutants/atwsmod.html for details.

[118] Jim Morris. "Lost Opportunity: EPA had its chance to regulate hydrogen sulfide." November 8, 1997. *The Houston Chronicle.*

[119] As quoted in *The Houston Chronicle.*

[120] Jim Morris, *The Houston Chronicle.*

[121] Environmental Protection Agency, Chemical Emergency Preparedness and Prevention. Look for $H_2S$ on the list at http://yosemite.epa.gov/oswer/ceppoehs.nsf/Alphabetical_Results!OpenView&Start=146

environment upon accidental release."[122] This classification requires companies that produce the substance to develop plans to prevent and respond to accidental releases. Importantly, however, this classification does not require regular emission controls of the substance.[123] Additionally, $H_2S$ is not on the list of toxic substances whose releases companies are required to report under the EPA's Toxic Release Inventory (TRI).[124] This exclusion is due to an administrative stay put in place on August 22, 1994, as a result of lobbying by a paper, forest, and wood products industry association.[125] The administrative stay will remain in effect until the EPA decides to lift it.

At the time of writing, the EPA is considering whether to re-evaluate including hydrogen sulfide on the HAPs list of the Clean Air Act.[126] The EPA is motivated by some concerns regarding chronic and acute exposure to hydrogen sulfide.[127] Further, if they proceed with research, the EPA's findings may inform action on the current administrative stay that is responsible for exempting $H_2S$ from TRI reporting requirements.[128]

The EPA does, however, have an inhalation reference concentration (RfC) for hydrogen sulfide, which is "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a

---

[122] Section 112(r) of the Clean Air Act, as cited in EPA, "Report to Congress on Hydrogen Sulfide Air Emissions," p.i.
[123] Jim Morris, *The Houston Chronicle*.
[124] EPA's Toxic Release Inventory list of chemicals for Reporting Year 2004. Available at http://www.epa.gov/tri/chemical/RY2004ChemicalLists.pdf
[125] See http://www.epa.gov/tri/guide_docs/2001/brochure2000.pdf, footnote on p.18.
[126] Personal communication with Jim Hirtz, February 24, 2006. US EPA, Health and Environmental Impacts Division, Research Triangle, North Carolina. The EPA undertook this action in response to a request by an environmental organization from Texas.
[127] Personal communication with Jim Hirtz, February 24, 2006. US EPA, Health and Environmental Impacts Division, Research Triangle, North Carolina.
[128] Personal communication with Jim Hirtz, March 2, 2006. US EPA, Health and Environmental Impacts Division, Research Triangle, North Carolina.

lifetime."[129] The RfC is one important standard for chronic exposure. According to the EPA's on-line Integrated Risk Information System (IRIS) database, the current inhalation RfC for hydrogen sulfide is $2x10^{-3}$ mg/m$^3$ (1.4 ppb). Applying the RfC definition, this means that it is possible that inhaling more than this concentration on a daily basis over a lifetime poses "an appreciable risk of deleterious effects." The RfC is well below any occupational standards set by OSHA or recommended by NIOSH and the ACGIH

The EPA also recommends levels of hydrogen sulfide for their Acute Exposure Guideline Levels (AEGL) for various exposure periods. These threshold exposure limits apply to the general public for emergency exposures ranging from 10 minutes to 8 hours, and are "intended to describe the risk to humans resulting from once-in-a-lifetime, or rare, exposure to airborne chemicals."[130] Appendix A includes definitions of the AEGL categories, and the recommended $H_2S$ levels for each exposure period and AEGL category.

Other guidelines also exist for exposure to hydrogen sulfide in emergency situations. To protect the health of the general public in the event of an emergency release, the American Industrial Hygiene Association (AIHA) establishes Emergency Response Planning Guidelines (ERPGs), which specify one-hour exposure limits. These limits are also included in the table in Appendix A.

The National Research Council's Committee on Toxicology recommended Emergency Exposure Guidance Level (EEGL) to the Department of Defense for

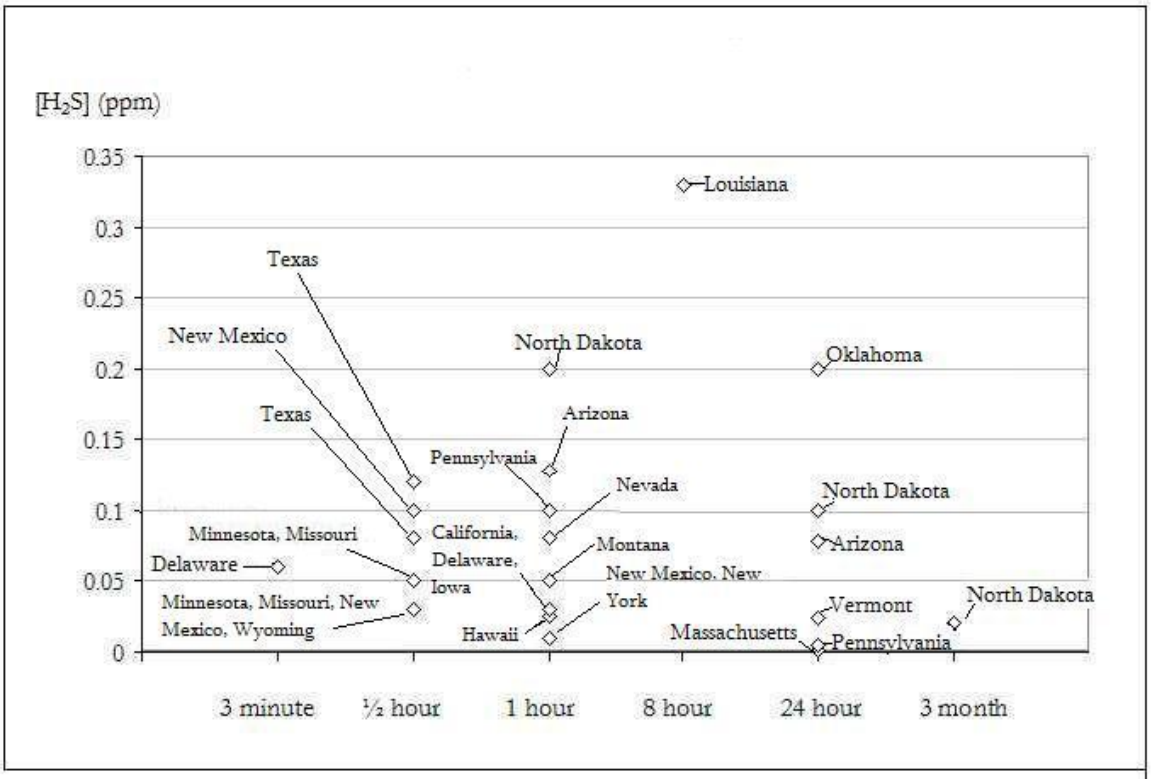
[129] EPA Integrated Risk Information System, Hydrogen sulfide (CASRN 7783-06-4), http://www.epa.gov/iris/subst/0061.htm.
[130] EPA, The Development of Acute Exposure Guideline Levels (AEGLs), http://www.epa.gov/oppt/aegl/index.htm

maximum concentrations acceptable in rare situations such as spills and fires.[131] The EEGLs apply to young and healthy military personnel, and exist for 41 substances, of which hydrogen sulfide is one. The 10 minute EEGL for $H_2S$ is 50 ppm, and the 24 hour $H_2S$ EEGL is 10 ppm.[132]

*6.2 State Regulations*

In the absence of federal standards for ambient levels of hydrogen sulfide, twenty states have passed their own laws to regulate $H_2S$ emissions. Figure 2 is a snapshot of state ambient hydrogen sulfide regulations. It illustrates the wide range of existing state standards.

**Figure 2: State Ambient $H_2S$ Regulations**




[131] National Oceanic and Atmospheric Administration, Office of Response and Restoration, "Public Exposure Guidelines" at http://archive.orr.noaa.gov/cameo/locs/expguide.html
[132] As cited in the EPA "Report to Congress on Hydrogen Sulfide Emissions," p.III-14.

A detailed table listing the states with ambient $H_2S$ standards can be found in Appendix B. States set their standards based on a variety of justifications, and if available, these are also listed in Appendix B. I compiled this data by reviewing information available on each state environmental department's website, and by speaking with appropriate staff. Some states have based their ambient standard for hydrogen sulfide on odor thresholds, while others have based their standard on health considerations, either adopting the EPA's RfC inhalation guideline, modifying the OSHA safety standard to apply to continuous exposure, or basing their standard on other health studies. The fact that these states have taken the initiative to regulate ambient $H_2S$ indicates that there is concern for human health even at these relatively low levels.

Many states' health/environmental departments routinely receive odor complaints about hydrogen sulfide. Specifically, staff at agencies in Colorado, Idaho, Iowa, Kansas, Michigan, Montana, Nevada, New Mexico, Ohio, Oregon, Texas, and Wyoming reported receiving many $H_2S$ odor complaints. In Kansas and Ohio, people have also complained about health effects from hydrogen sulfide. In Colorado, there have been some cattle deaths attributed to exposure to hydrogen sulfide, which had collected in low-lying areas.

In addition to inquiring about ambient hydrogen sulfide standards, I collected information about any monitoring of $H_2S$ – routine or otherwise – that the state agency conducts. The most frequently cited reason for the lack of routine monitoring, even in states with ambient $H_2S$ standards, are budget constraints. A number of people said that monitoring and more information in general would be desirable. Some states have conducted periodic, project-based monitoring of hydrogen sulfide. Studies of hydrogen sulfide emissions from Arkansas, Colorado, Louisiana, New Mexico, and North Dakota

are available. These studies are of varying quality and scope, but each sheds some light on the topic of hydrogen sulfide emissions and oil and gas operations.

### *6.2.1 Special $H_2S$ Monitoring Studies*

#### *6.2.1.1 Arkansas*

The Arkansas Department of Environmental Quality conducted two hydrogen sulfide monitoring studies in response to numerous health and welfare related concerns of Texarkana residents about emissions from gas processing plants in the area.[133] The first study, spanning 1995 to 1997, was a scoping study to determine whether hydrogen sulfide was indeed present in ambient air and to determine whether the facilities that were emitting $H_2S$ were in compliance with their emissions permits. After this study established that $H_2S$ was present in the air, a second, more rigorous study was conducted from March 1998 through March 1999. The state does not have an ambient hydrogen sulfide standard.

The monitoring data from the latter study has been reported to the EPA's Air Quality System (AQS) database. The AQS database contains measurements of air pollutants – criteria pollutants, hazardous air pollutants, and other monitored substances – and this data is publicly available.[134] The Arkansas Department of Environmental Quality itself did not provide any monitoring data or comments. Data from the AQS site[135] is available for two monitoring locations, which are classified as rural residential. At the first monitoring location, the mean concentrations for the monitoring periods from May to

[133] Pleasant Hills $H_2S$ Study, obtained February 2006 by mail from Jay Justice, Senior Epidemiologist with the Arkansas DEQ.
[134] http://www.epa.gov/air/data/aqsdb.html
[135] http://oaspub.epa.gov/aqspub1/aqs_query.psite The code for hydrogen sulfide is 42402.

July 1998, and October to December 1998, were 2.4 ppb and 3.4 ppb, respectively, and the maximum hydrogen sulfide concentrations were 35 ppb and 24 ppb, respectively. The levels of $H_2S$ recorded at the second monitoring location for which data is available on the AQS site were slightly higher than at the first. The mean concentration in December 1998 was 4 ppb, and in January 1999, 5.5 ppb. The maximum concentration recorded in those months were 55 ppb and 127 ppb, respectively. These levels of hydrogen sulfide, while not very high, are nevertheless higher than normal urban background levels of up to 0.33 ppb.[136] The levels measured in this study may be expected to produce a persistent odor, which has been shown in one study (Schiffman et al., 1995) to have a negative effect on the mood of nearby residents. Based on the literature reviewed above, there is little evidence of more serious health effects attributable to these levels of $H_2S$.

*6.2.1.2 Colorado*

In 1997, the Colorado Department of Public Health and Environment (CDPHE), Air Pollution Control Division, conducted a monitoring study of $H_2S$ concentrations near several known sources, and of urban and rural background ambient levels.[137] The CDPHE initially considered monitoring at oil and gas sites because of the information in the 1993 EPA report on emissions of $H_2S$ at points of oil and gas extraction. Ultimately, the Colorado study excluded oil and gas operations, because of assurances from the Colorado Oil and Gas Conservation Commission (COGCC) that elevated $H_2S$ levels are not

[136] Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Toxicological profile for hydrogen sulfide (*Draft for Public Comment*). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Chapter 2, p.1.
[137] "Hydrogen Sulfide Concentrations in Colorado; Results from a Screening Survey." Prepared by The Technical Services Program, Air Pollution Control Division, Colorado Department of Public Health and the Environment, 1997. Obtained February 2006 by mail from Ray Mohr, CDPHE.

common in deposits in Colorado.[138] However, interviews with people living near oil and gas sites in Colorado, presented below, suggest that hydrogen sulfide is present near these facilities. The COGCC itself has not conducted any monitoring of $H_2S$ at oil and gas sites. Thus, the question of what concentrations of hydrogen sulfide are present near oil and gas operations in the state is still unanswered. Colorado does not have an ambient hydrogen sulfide standard.

#### *6.2.1.3 Louisiana*

The Louisiana Department of Environmental Quality, motivated by numerous odor complaints from nearby residents, monitored hydrogen sulfide and sulfur dioxide concentrations downwind of the Calumet Refinery in Shreveport.[139] The hourly average concentration for hydrogen sulfide, for the monitoring period from October 2002 to April 2005, was 2.56 ppb, with a maximum of 50.15 ppb and a median of 1.92 ppb.[140] These measurements correspond to the range of the monitoring data from Arkansas, and the same analysis of potential health effects applies.

#### *6.2.1.4 New Mexico*

In February 2002, the Air Quality Bureau of the New Mexico Environment Department monitored hydrogen sulfide levels to determine if ambient concentrations near certain facilities are in compliance with the state's ambient standards.[141] Air samples

---

[138] "Hydrogen Sulfide Concentrations in Colorado," p.2.
[139] James M. Hazlett, "Report for the Calumet Air Monitoring Project," Louisiana Department of Environmental Quality, Office of Environmental Assessment. June 8, 2005. (obtained from the author and used with permission.)
[140] Hazlett, p.4.
[141] New Mexico Environment Department (NMED), Air Quality Bureau. "Trip Report: $H_2S$ Survey, March 18-22, 2002." By Steve Dubyk and Sufi Mustafa. Obtained from the author.

were collected near a sewage treatment plant, four dairy operations, a poultry operation, one liquid septage facility, one sewage sludge disposal facility, and several oil and gas facilities.[142] Table 3 presents the data from the monitors near the oil and gas facilities, and a discussion of the results follows.

**Table 3: Summary of Monitoring Data from New Mexico Study**

| **Facility type** | **$H_2S$ concentration measured at monitoring site (ppb)[143]** | |
|---|---|---|
| | **Range** | **Average** |
| Indian Basin Hilltop, no facility | 5 – 8 | 7 |
| Indian Basin Compressor Station | 3 – 9 | 6 |
| Indian Basin Active Well Drilling Site | 7 – 190 | 114 |
| Indian Basin Flaring, Production, and Tank Storage Site | 4 – 1,200 | 203 |
| Marathon Indian Basin Refining and Tank Storage Site | 2 – 370 | 16 |
| Carlsbad City Limits, near 8 to 10 wells and tank storage sites | 5 –7 | 6 |
| Carlsbad City Limits, Tracy-A | 5 – 8 | 7 |
| Compressor station, dehydrators – Location A | 4 –5 | 4 |
| Compressor station, dehydrators – Location B | 2 – 15,000 | 1372 |
| Huber Flare/Dehydrating Facility [a] | 4 – 12 | 77 |
| Snyder Oil Well Field | 2 – 5 | 4 |
| Empire Abo Gas Processing Plant | 1 – 1,600 | 300 |
| Navajo Oil Refinery | 3 – 14 | 7 - 8 |

[a] Strong winds, flare not operating correctly at time of sampling may have caused lower readings than expected, according to study, p.8.

The New Mexico data indicates that ambient concentrations of hydrogen sulfide at the sampling locations, which included both oil and gas facilities and sites without oil and gas facilities, are at least an order of magnitude greater than 0.11 to 0.33 ppb, which are the ambient levels of $H_2S$ that can normally be expected in urban areas.[144] The ambient levels recorded at the two sites without expected sources of $H_2S$ – Indian Basin Hilltop, no facility and Carlsbad City Limits, Tracy-A – both averaged 7 ppb, indicating that usual

[142] NMED Trip Report, p.1.
[143] The monitor that the NMED used recorded hydrogen sulfide concentrations every 30 seconds for 3 minutes. The averages reported in this table are averages of 3-minute mean concentrations.
[144] Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Toxicological profile for hydrogen sulfide (*Draft for Public Comment*). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Chapter 2, p.1.

$H_2S$ concentrations in this part of New Mexico are higher than normal urban background levels.

Hydrogen sulfide levels sampled at flaring, tank storage, and well drilling sites, averaging from approximately 100 to 200 ppb, are significantly elevated compared to normal background levels, and compared to usual background $H_2S$ concentrations in this area of New Mexico. While these concentrations generally produce a nuisance due to odors which may translate into headaches, nausea and sleep disturbances if exposure is constant, one study discussed above (Legator et al., 2001) found central nervous system, respiratory system, and ear, nose and throat symptoms associated with annual average hydrogen sulfide levels ranging from 7 to 27 ppb. Overall, the data shows that concentrations of $H_2S$ vary widely, even at similar facilities: at one compressor / dehydrator, the average concentration over the course of monitoring was 4 ppb, while at another, the average was 1372 ppb. The data further demonstrates that $H_2S$ is present, often at quite elevated levels, at oil and gas facilities. A staff person at the NMED indicated that there is need for more monitoring and a better-designed study, but that budget constraints prevent them from routine monitoring. The department had rented a hydrogen sulfide monitor for this study.

*6.2.1.5 North Dakota*

The North Dakota State Department of Health and Consolidated Laboratories monitored hydrogen sulfide emissions from oil and gas wells at several locations, from 1980 until 1992. Each location was near at least one oil or gas well. At one location, the Lostwood Wildlife Refuge monitoring station, the highest one hour average concentration

recorded was 88 ppb, in 1990.[145] At Lone Butte, 6 miles north of the Theodore Roosevelt National Park, one hour average hydrogen sulfide concentrations frequently exceeded 200 ppb.[146] At another site, in a valley with several wells within one mile from the monitor, recorded concentrations were as high as 250 ppb.[147] These findings highlight the fact that hydrogen sulfide is routinely emitted near oil and gas wells.

These monitoring studies reveal that hydrogen sulfide is present at oil and gas facilities, including oil refineries, gas processing plants, oil and gas wells, flares, and compressor stations. These types of facilities are commonly situated near residences, where people can be routinely exposed to hydrogen sulfide. The levels of $H_2S$ range from relatively low concentrations of 2 ppb recorded in Louisiana to the much higher concentrations observed in New Mexico and North Dakota.

### *6.2.2 Routine Monitoring*

Of the twenty states that have an ambient hydrogen sulfide standard, only three – California, Oklahoma, and Texas – conduct routine monitoring of ambient $H_2S$ concentrations. The other eighteen states do not monitor ambient $H_2S$ levels. Rather, the standard is generally used in permitting facilities that emit hydrogen sulfide. Typically, the health/environmental departments model emissions and permit a facility if the model reports that the emissions would not raise ambient levels above the standard.

#### *6.2.2.1 California*

The California Air Resources Board (CARB), which manages air quality and pollution in the state, has authority to enforce the state ambient hydrogen sulfide standard

[145] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-22.
[146] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-26.
[147] EPA, "Report to Congress on Hydrogen Sulfide Emissions," p.III-30.

of 30 ppb, averaged over one hour. CARB also delegates management to the state's 35 Air Pollution Control Districts (APCDs) or Air Quality Management Districts (AQMDs), each with authority to adopt its own rules and regulations to control and monitor emissions of hydrogen sulfide. A map of the state air districts is in Appendix C. The local districts defer to the state ambient standard, but they are in charge of conducting monitoring of ambient $H_2S$.

The twelve sites in California where hydrogen sulfide is routinely monitored were chosen because of nearby emission sources. Table 4 summarizes the monitoring sites and the sources of $H_2S$. I discuss the data for 2005 from Contra Costa and Santa Barbara Counties, where the $H_2S$ sources are due to oil and gas facilities. Daily averages of hourly hydrogen sulfide readings at the three monitoring sites in Contra Costa County range from 0.000 to 0.003 ppm, with one reading of 0.007 ppm at one monitoring site. Similarly, the daily averages of hourly $H_2S$ concentrations recorded during 2005 at all three sites in Santa Barbara range from 0.000 to 0.001 ppm.[148] These levels are most likely of no health concern.

**Table 4: California $H_2S$ Monitoring Sites**

| District | County | Sites | Source(s) |
|---|---|---|---|
| Great Basin Unified APCD | Inyo | 2 | Geothermal Power Plant |
| Lake County AQMD | Lake | 3 | Geothermal Power Plants |
| Mojave Desert AQMD | San Bernardino | 1 | Chemical Processing Facility |
| San Francisco Bay Area AQMD | Contra Costa | 3 | Chevron Oil Refinery |
| Santa Barbara County APCD | Santa Barbara | 3 | Oil and Gas Processing Facilities |

[148] Data is available at http://www.arb.ca.gov/adam/cgi-bin/db2www/adamweeklyc.d2w/start. In Step 3, select desired county, and on the next page, in Step 1, select "Daily Average of Hourly Measurements." Use arrows on the right to select different time periods.

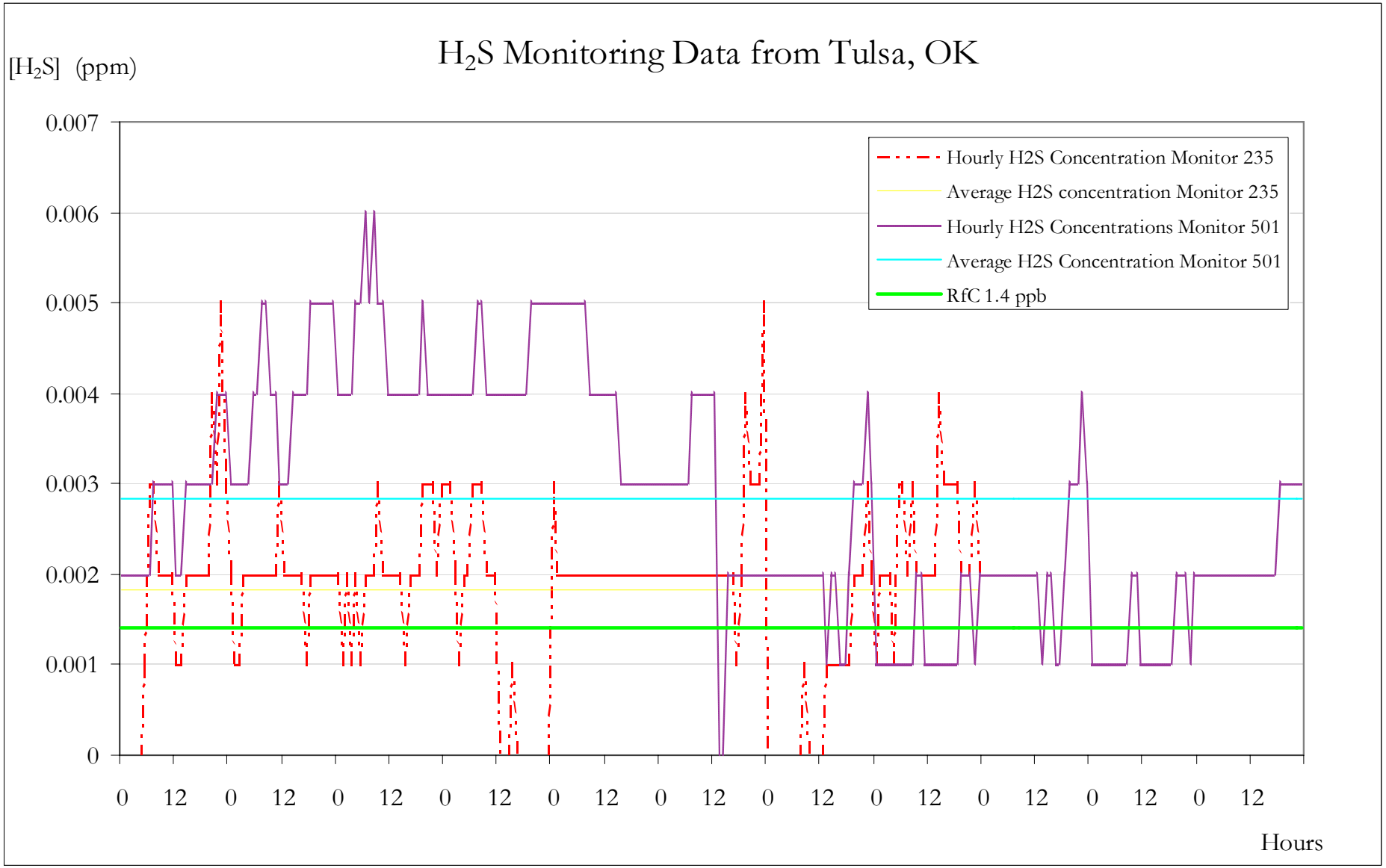
*6.2.2.2 Oklahoma*

The Air Quality Monitoring division of the Oklahoma Department of Environmental Quality (DEQ) continuously monitors ambient levels of hydrogen sulfide at sites downwind of two large oil refineries in Tulsa. The DEQ initiated the monitoring because complaints about foul odors numbered as many as 5 or 6 per day.[149] According to staff at the Oklahoma DEQ, the DEQ installed three monitors in Tulsa, and continuous hourly average data for two of the three monitors is available on-line.

Figure 3 summarizes the data on ambient $H_2S$ levels recorded at these two sites in Tulsa. Monitor 235 is in a park right next to residences an eighth to a quarter of a mile downwind and across the river from a refinery. Monitor 501 is on a hill, two to three miles downwind of another refinery. The hill elevation approximately lines up with the height of the refinery stacks. The majority of the odor complaints mentioned above came from residents of this neighborhood. Now, the DEQ receives about 3 or 4 complaints a week. The levels of hydrogen sulfide in both neighborhoods, although not very high, are nevertheless above the EPA's RfC of 1.4 ppb, and are well elevated above normal background levels of 0.11 to 0.33 ppb. It is possible that continuous exposure to these levels poses health risks. While the Oklahoma DEQ is monitoring hydrogen sulfide levels, there is no concurrent community health or exposure study investigating the health effects of chronic exposure to these levels of $H_2S$.

[149] Personal communication, Rhonda Jeffries, Oklahoma Department of Environmental Quality. February 10, 2006.

**Figure 3: Tulsa $H_2S$ Monitoring Data**[150]




[150] Data from http://www.deq.state.ok.us/aqdnew/monitoring/cpdata.htm#

*6.2.2.3 Texas*

The Texas Commission on Environmental Quality (TCEQ) coordinates a network of monitors across the state to insure compliance with its ambient air quality standards. Hydrogen sulfide is among the pollutants that are routinely monitored. There are twelve active $H_2S$ monitors in Texas, some in residential and some in industrial areas, each near an oil or gas facility, including a refinery, a tank battery, and a compressor station.[151] The majority of the monitors register relatively low $H_2S$ levels, ranging from 0.1 ppb to 5 ppb. However, one monitor at compressor station near a residence, consistently records elevated levels of $H_2S$. In December 2005, the last month for which the data has been validated by the TCEQ, 20 percent of the hourly readings exceeded the state standard of 0.8 ppm.[152] Chronic exposure to such levels, generally considered a nuisance due to odor, has also been shown to adversely affect human health, as discussed in the Literature Review – Chronic Exposure section.

## 7. Evidence From People Living Near Oil and Gas Operations

I conducted semi-structured, informal telephone interviews with people who believe that their health has been compromised as a result of exposure to hydrogen sulfide from oil and gas operations. Appendix D contains narratives of each interview, and provides details about each interviewee's experience. Some interviewees had previously contacted the Oil and Gas Accountability Project (OGAP), a non-profit organization working to reduce the impacts of oil and gas development on communities and the

[151] Data and photos are available at http://www.tceq.state.tx.us/compliance/monitoring/air/monops/sites/monitors_map.html

[152] 65 out of 332 readings were above 80 ppb. (40 data points did not include readings due to equipment maintenance).

environment, about their concerns. These interviewees identified other potential interview participants. I spoke with eleven people, and obtained information about the experiences of two additional people who lived with the interviewees. Thus, the information I present here is based on the experience of thirteen people. The ages of the interview participants range from 25 to 76. To protect the privacy of the interviewees, their names and other identifying characteristics have been withheld.

Table 4 summarizes the information on the sources of $H_2S$, lengths of exposure, and symptoms reported by each interviewee. Hydrogen sulfide exposure was due to emissions from a variety of sources. As identified in the interviews, these consisted of a natural gas sweetening facility, natural gas and oil well sites, flaring operations at both oil and gas facilities, venting, wastewater pits, and an oil refinery. The duration of exposure also varied, from one year to as long as eleven years, and these exposure periods in several cases include instances of acute exposure to accidental high-concentration $H_2S$ releases. Some interviewees had information on the concentrations of $H_2S$ to which they were exposed, while others did not.

The reported health effects are consistent with exposure to hydrogen sulfide, and include both physical and neurological symptoms. The most commonly reported symptoms were pressure headaches or dull headaches (ten people), fatigue or loss of energy (seven people), and memory impairments (seven people). Dizziness, throat irritation, eye irritation, heart palpitations, and insomnia were each reported by four people, and nosebleeds by five people. Other symptoms that the interviewees are experiencing are balance problems, trouble walking, vomiting, coughing, concentration problems, skin irritations (in some cases severe), and shortness of breath.

Eight people are experiencing chemical hypersensitivity and attribute it to hydrogen sulfide exposure. Two people I interviewed have had their gall bladders removed, which may or may not have been related to hydrogen sulfide exposure, and four have been diagnosed with chemical encephalopathy (swelling of the brain). Three interviewees are on permanent Social Security disability as a result of their health problems, which they attribute to hydrogen sulfide exposure. Five separate interviewees also stated that animals in their area were experiencing health problems. Every interviewee reported that the characteristic rotten egg odor of hydrogen sulfide was commonplace at their residences. Four families that I interviewed chose to move from their previous residences because of the health problems they associate with hydrogen sulfide.

While some of the interviewees have been diagnosed by medical professionals who attributed their symptoms to exposure to hydrogen sulfide, others have not. Oil and gas operations emit a host of other pollutants in addition to hydrogen sulfide, many of which are hazardous to human health, confounding the process of ascribing health effects to just one chemical. Additional confounders are individual health factors and the potential presence of other sources of hydrogen sulfide. This difficulty of disaggregating pollutants and symptoms provides an opportunity for critics to undermine the conclusion that the health problems reported by the people I interviewed are due to hydrogen sulfide. Nevertheless, the symptoms that the interviewees experienced match the health effects associated with hydrogen sulfide exposure as reported in the toxicological studies I present above.

The interviews provide evidence, which, although it is anecdotal, attests to the fact that hydrogen sulfide is emitted at a host of oil and gas facilities, and that its continual presence in ambient air compromises human health and well being. Although the concentrations of $H_2S$ to which the interviewees were exposed are, for the most part, not known, they likely are not very high. Except for the three cases of knockdown, the interviews show that chronic exposure to relatively low levels of hydrogen sulfide can nevertheless take a considerable toll on people's health. The health problems that the people I interviewed are experiencing are serious enough to warrant monitoring of ambient air near oil and gas facilities in residential areas to ensure that $H_2S$ levels are not above those considered safe. Clearly, the very issue of what levels of hydrogen sulfide are "safe" is contentious, judging in part by the wide range of values that characterize states' ambient $H_2S$ standards. More research is certainly needed, both to determine the effects of chronic exposure to low levels of $H_2S$ and to establish a sound standard for safe exposure to $H_2S$.

**Table 4: Summary of Interviews**

| | Age and Sex | Source of Emissions | Exposure Time and Concentrations | Symptoms | Diagnosis |
|---|---|---|---|---|---|
| Story 1 | Male 76 | Venting from tank battery | 3 years ongoing, 2 acute exposures | *Ongoing*: Staggering, dizziness, pressure headaches, dry cough, shortness of breath, throat irritation, fatigue, lightheadedness, insomnia, lack of concentration, memory loss;<br><br>*Acute exposure*: stiff neck, dizziness and dry heaving | chemical encephalopathy chemical hypersensitivity due to $H_2S$ |
| | Male 25 | Sour gas well, tank battery | Ongoing; one acute exposure- knockdown, 200 ppm | *Knockdown*: severe headache<br><br>*Persistent symptoms*: very bad memory, some balance problems | |
| Story 2 | Female 44 | Oil refinery, waste water treatment plant, oil pads | Ongoing, unknown concentrations | Headaches, balance problems, concentrations problems, eye irritation, problems sleeping, general pain, low muscle strength, trouble walking, problems with memory retention and reading comprehension, hypersensitivity of the skin | chemical encephalopathy |
| Story 3 | Male and Female, mid-40s | Natural gas well flaring | Ongoing, unknown concentrations | *Wife*: pains similar to severe rheumatoid arthritis, blistering of the skin when showering, severe burns on the bottoms of her feet, skin covered in welts, fatigue, vomiting, rectal bleeding, severe sinus headaches; gall bladder removed<br>*Husband*: rectal bleeding | None specific to H2S |
| Story 4 | Male and Female, mid-60s | Amine plant, sour gas wells | Ongoing for 1 year; unknown concentrations | *Both*: chronic sore throat, congestion, coughing, headaches, swollen eyes, insomnia, occasional nosebleeds, and a general lack of energy.<br>*Husband*: face burns, burning on hands and eyes.<br>*Wife*: heart palpitations | None |
| Story 5 | Male and female, mid-40s | Oil refinery, waste water treatment | Husband: occupational; ongoing residential Wife: ongoing; Concentrations 3 ppm; 3 or 4 times per week 10 ppm | *Husband*: bloody nose, headaches, colon polyps, burning eyes, throat itching, and itching all over, severe headaches, severe rashes, burning skin, teeth damage, nerve damage, slurred speech; on disability, unable to work<br>*Wife*: rapidly deteriorating eyesight, ringing in her ears, memory problems, trouble with balance, tremors, trouble walking up and down stairs, and severe migraine headaches; gall bladder removed | chemical encephalopathy |
| Story 6 | Female 50s | Sour natural gas wells flaring, tank battery | Ongoing; one acute exposure | *Acute exposure*: headache, extremely sick; *Later*: extreme fatigue, confusion, anxiety, heart symptoms, shaking and tremors when exposed to certain chemicals, dizziness, headaches, nosebleeds, memory and cognitive impairments, especially upon exposure to H2S or other chemicals, bronchial asthma symptoms<br>*Son knocked down*: arrhythmia, balance problems, and pneumonia; *Currently:* nosebleeds, walks with a cane, experiences extreme headaches, confusion, brain fog, ongoing heart problems, burning lungs when he's in an area with chemicals, scaling of the skin, psoriasis, and chloro-acne | chemical encephalopathy, non-recoverable |

| | | | | | |
|---|---|---|---|---|---|
| Story 7 | Male 27 | Gas processing plant, wells | Ongoing, one acute exposure | Acute exposure: extreme shortness of breath, close to unconsciousness<br>Ongoing: blurred vision, brain fog, memory impairment, excessive sleepiness, lack of energy and strength, occasional diarrhea, blood in his urine, loss of libido, abnormal heart rhythm, and anxiety-like attacks, severe and protracted involuntary muscle movements in his arms and legs. | Chemical encephalopathy, optical nerve damage |
| Story 8 | Female 50s | Gas wells, flares, condensate pits | Ongoing occupational, one acute exposure | Acute exposure: knockdown, blinding headache, fading of consciousness<br>Ongoing: nasal irritation, balance and memory impairment, nosebleeds, nerve inflammation | Chemical sensitivity |

## 8. Concluding Remarks

The literature on human health and hydrogen sulfide reveals serious and lasting physiological and neurological effects associated with acute exposure. The health effects of chronic exposure to lower levels of $H_2S$, as documented in several studies, also include persistent physiological and neurological disturbances. Oil and gas facilities can be expected to accidentally and routinely emit hydrogen sulfide in concentrations that span a wide range and are associated with a variety of health effects. Academic studies, my conversations with health department staff, and available data from monitoring projects help establish that hydrogen sulfide is indeed present near oil and gas facilities.

Because people live near oil and gas sites, emissions of $H_2S$ may be routinely compromising human health. The interviews I conducted with people who live close to oil and gas facilities, as well as some research reported in the Literature Review section, provide evidence of health impacts from exposure to $H_2S$ emitted by oil and gas development. Although the anecdotal evidence from my interviews is vulnerable to criticism that other pollutants or individual health factors may be responsible for the symptoms, the reported health effects are consistent with hydrogen sulfide exposure. The fact that concentrations of $H_2S$ to which people are exposed are often not known does not imply that hydrogen sulfide is not the cause of the observed health effects. The lack of precise exposure data is, however, one area that future research should address.

In the meanwhile, people's health needs to be protected. The proximity of oil and gas wells to people's residences is one route of exposure to hydrogen sulfide, and to other pollutants associated with oil and gas extraction. The persistence of the land ownership pattern known as 'split estate,' under which one entity owns the rights to the surface of the property and another to

the minerals under the surface, is partly responsible for the proximity of oil and gas facilities to residences. Another factor are low setbacks, the minimum distance required between an energy facility and a specific type of development.[153] For example, in Colorado, where some of the interviewees live, the residential setback requirement for oil and gas wells is 150 feet.[154] In Texas, the setback is also 150 feet,[155] while the New Mexico residential setback is just 100 feet.[156] In Alberta, Canada, the residential setback requirement for sour gas wells areas is 100 m (approximately 330 feet).[157] While greater than Colorado's and Texas's required setback, this distance may not be sufficient, as some of the interviewees were exposed to hydrogen sulfide in Alberta. To truly provide a margin of safety and protection to people who live in areas of oil and gas development, whether the facilities are on their surface property or not, greater setback distances need to be established. The siting of oil refineries and gas processing plants near residences, and conversely, building homes near existing refineries and gas plants, exposes people to a host of pollutants, including hydrogen sulfide. This is often an issue with the dimension of social and environmental justice added to questions of protecting public health.

Some technological options exist that may help mitigate the effects of hydrogen sulfide on the health of people who live near emission sources. One advanced technology for odor control, consisting of a dry scrubbing system with multiple beds of engineered media (made by soaking, or on a rotating agglomeration disk), removed hydrogen sulfide at a wastewater

---

[153] http://www.eub.ca/portal/server.pt/gateway/PTARGS_0_0_257_229_0_43/http%3B/extContent/publishedcontent/publish/eub_home/public_zone/eub_process/enerfaqs/EnerFAQs5.aspx#1

[154] Colorado Oil and Gas Conservation Commission, Rule 603. Available at http://oil-gas.state.co.us/RR_Asps/600Series.pdf

[155] Texas Administrative Code, Title 16, Part I, Chapter 3, Rule 3.21 (a) and (i). Available at http://info.sos.state.tx.us/pls/pub/readtac$ext.ViewTAC?tac_view=4&ti=16&pt=1&ch=3&rl=Y

[156] Personal communication, Denny Foust, New Mexico Environment Department, April 12, 2006.

[157] Alberta Energy and Utilities Board, Directive 056, Energy Development Applications and Schedules. Available at http://www.eub.ca/docs/documents/directives/directive056.pdf, pp.54-55.

treatment facility with an efficiency of 99.94 percent.[158] This odor control technology reduced the peak inlet hydrogen sulfide concentration of 108.0 ppm to 0.061 ppm.[159] Such odor abatement technologies could be required at all facilities that emit hydrogen sulfide, including oil refineries and gas processing plants. At points of oil and gas extraction and processing, requiring high efficiency flares would ensure that less hydrogen sulfide (and other pollutants) escape into ambient air unburned.

As I show in the Regulations and Recommendations section, at the federal level, the oil and gas industry and the paper and pulp industry have exerted their influence to prevent $H_2S$ from being included on the Clean Air Act's Hazardous Air Pollutants (HAPs) list, and to exempt it from reporting under the EPA's Toxic Release Inventory (TRI). At the time of writing, the EPA is reviewing both decisions, which at the very least indicates that some concern exists over the lack of stricter regulation of hydrogen sulfide at the federal level. The level of regulation of hydrogen sulfide varies widely across the states that have established an ambient standard in the absence of a federal one, but again, the very existence of ambient standards suggests that hydrogen sulfide is a concern.

Monitoring of ambient $H_2S$ is necessary to determine exactly how much is being emitted and to clarify the link between exposure and health effects. Enough evidence of routine $H_2S$ emissions at oil and gas facilities emerges from my conversations with health department personnel, interviews with people living near oil and gas sites, several studies summarized in the Literature Review section, and state monitoring projects to merit more comprehensive monitoring. The lack of federal standards for ambient $H_2S$ levels or for emissions of $H_2S$ is one reason for sparse monitoring even at state level, since state health / environmental departments

[158] Gailey, Karen. 2004. "Goodbye Odors, Hello Happy Neighbors." *Pollution Engineering*. p.30.
[159] Gailey, Karen. 2004. "Goodbye Odors, Hello Happy Neighbors." *Pollution Engineering*. p.30.

largely depend on federal funding for their projects. More routine and special project monitoring would facilitate conducting community health studies, by providing accurate exposure data that could be matched with observed health effects.

In light of the information presented here on the health effects associated with exposure to hydrogen sulfide, even though rigorous data on the dose-response relationship is lacking, it is irresponsible and callous to delay making some public policy decisions that would help protect human health.

**Bibliography**


Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Toxicological profile for hydrogen sulfide (*Draft for Public Comment*). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Bates, Michael, Nicholas Garrett, et al. 1998. "Cancer incidence, morbidity and geothermal air pollution in Rotorua, New Zealand." *International Journal of Epidemiology*. **27**:10-14,

Bates MN, Garrett N, Shoemack P. 2002. "Investigation of health effects of hydrogen sulfide from a geothermal source." *Archives of Environmental Health*, **57**:405- 411.

Beauchamp, R.O. et al. 1984. "A Critical Review of the Literature on Hydrogen Sulfide Toxicity." *Critical Reviews in Toxicology*. **13**: (1) 25-97.

Brown, Kenneth G. and Gary L. Foureman. 2005. "Concentration-time-response modeling for acute and short-term exposures." *Regulatory Toxicology and Pharmacology*. **43**: 45-54.

Collins, James and David Lewis, Air Toxicology and Epidemiology Section, California Office of Environmental Health Hazard Assessment (OEHHA). September 1, 2000. *Hydrogen Sulfide: Evaluation of Current California Air Quality Standards with Respect to Protection of Children.* Prepared for California Air Resources Board, CA OEHHA.

Converse, Jean, and Stanley Presser. 1986. *Survey Questions: Handcrafting the Standardized Questionnaire*. Sage University Paper series on Quantitative Applications in the Social Sciences, Series no. 07-063. Beverly Hills, CA: Sage Publications.

Cottle, Merva K.W. and Tee L. Guidotti, M.D. 1990. "Process Chemicals in the Oil and Gas Industry: Potential Occupational Hazards." *Toxicology and Industrial Health*. **6**: (1) 41-56.

Steve Dubyk and Sufi Mustafa. "Trip Report: $H_2S$ Survey, March 18-22, 2002." New Mexico Environment Department, Air Quality Bureau.

Environmental Protection Agency. October 1993. "Report to Congress on Hydrogen Sulfide Air Emissions Associated with the Extraction of Oil and Natural Gas." EPA-453/R-93-045,

Fuller, Douglas C., MD, MPH, and Anthony J. Suruda, MD, MPH. September 2000. "Occupationally Related Hydrogen Sulfide Deaths in the United States From 1984 to 1994." *Journal of Occupational and Environmental Medicine*. **42**:(9) 939-942.

Gailey, Karen. 2004. "Goodbye Odors, Hello Happy Neighbors." *Pollution Engineering*. **36**: 28-30.

Glass, D.C. 1990. "A Review of the Health Effects of Hydrogen Sulphide Exposure." *Annals of Occupational Hygiene.* **34**: (3) 323-327.

Guidotti, Tee L. 1994. "Occupational exposure to hydrogen sulfide in the sour gas industry: some unresolved issues." *International Archives of Occupational and Environmental Health*. **66**: 153-160.

Hazlett, James M. June 8, 2005. "Report for the Calumet Air Monitoring Project," Louisiana Department of Environmental Quality, Office of Environmental Assessment.

Hessel, Patrick A., PhD. et al. 1997. "Lung Health in Relation to Hydrogen Sulfide Exposure in Oil and Gas Workers in Alberta, Canada." *American Journal of Industrial Medicine*. **31**:554-557.

Inserra, Steven, et al. 2002. "Community-based exposure estimate for hydrogen sulfide." *Journal of Exposure Analysis and Environmental Epidemiology*. **12**: 124-129.

Iowa State University and The University of Iowa Study Group. February 2002. *Iowa Concentrated Animal Feeding Operations Air Quality Study*.

Kilburn, Kaye H. and Raphael H. Warshaw. 1995. "Neurotoxic Effects from Residential Exposure to Chemicals from an Oil Reprocessing Facility and Superfund Site." *Neurotoxicology and Teratology*. **17**: (2) 89-102.

Kilburn, Kaye H. 1999. "Evaluating health effects from exposure to hydrogen sulfide: central nervous system dysfunction." *Environmental Epidemiology and Toxicology*. **1**:207-216.

Kilburn, Kaye H. 2003. "Effects of Hydrogen Sulfide on Neurobehavioral Function." *Southern Medical Journal*. **96**: (7) 639-646.

Kilburn, Kaye H. 2004. *Endangered Brains: How Chemicals Threaten Our Future*. Birmingham, AL: Princeton Scientific Publishers Company, Inc.

Knight, Laura D., MD, and S. Erin Presnell, MD. 2005. "Death by Sewer Gas: Case Report of a Double Fatality and Review of the Literature." *The American Journal of Forensic Medicine and Pathology*. **26**: (2) 181-185.

Layton, David W. and Richard T. Cederwall. 1986. "Assessing and Managing the Risks of Accidental Releases of Hazardous Gas: A Case Study of Natural Gas Wells Contaminated with Hydrogen Sulfide." *Environment International*. **12**: 519-532.

Layton, David W. and Richard T. Cederwall. 1987. "Predicting and Managing the Health Risks of Sour-Gas Wells." *Journal of the Air Pollution Control Association.* **37**: 1185-1190.

Legator, Marvin S., et al. March/April 2001. "Health Effects from Chronic Low-Level Exposure to Hydrogen Sulfide." *Archives of Environmental Health*. **56:** (2) 123-131.

McGavran, Pat. June 19, 2001. "Literature Review of the Health Effects Associated with the Inhalation of Hydrogen Sulfide." Idaho Department of Environmental Quality, Boise, Idaho.

McIntush, K.E., Dalrymple, D.A. and Rueter, C.O. July, 2001. "New process fills technology gap in removing H2S from gas," *World Oil*.

Milby, Thomas H. MD, and Randall C. Baselt, PhD. 1999. "Hydrogen Sulfide Poisoning: Clarification of Some Controversial Issues." *American Journal of Industrial Medicine*. **35**: 192-195.

Morris, Jim. November 8, 1997. "Lost Opportunity: EPA had its chance to regulate hydrogen sulfide." *The Houston Chronicle.*

Partti-Pellinen, Kirsi, Marttila Olli, Vilkka Vesa, et al. 1996. "The South Karelia Air Pollution Study: effects of low-level exposure to malodorous sulfur compounds on symptoms." *Archives of Environmental Health.* **51**. (4) 315-320.

Schiffman, Susan S., Elizabeth A. Sattely, et al. 1995. "The Effect of Environmental Odors Emanating From Commercial Swine Operations on the Mood of Nearby Residents." *Brain Research Bulletin*. **37**: (4) 369-375.

Snyder, Jack W., MD, PhD. et al. 1995. "Occupational Fatality and Persistent Neurological Sequelae After Mass Exposure to Hydrogen Sulfide." *American Journal of Emergency Medicine*. **13**: (2) 199-203.

Strickland, Judy A. and Gary L. Foureman. 2002. "US EPA's acute reference exposure methodology for acute inhalation exposures." *The Science of the Total Environment*. **288**: 51-63.

Tarver, Gary A. and Purnendu K. Dasgupta. 1997. "Oil Field Hydrogen Sulfide in Texas: Emission Estimates and Fate." *Environmental Science and Technology*. **31**: (12) 3669-3676.

Xu, Xiping, Sung-Il Cho, et al. 1998. "Association of petrochemical exposure with spontaneous abortion." *Occupational and Environmental Medicine*. **55**: 31-36.

## Appendix A: Guidelines for Occupational Exposure to Hydrogen Sulfide

| [$H_2S$] (ppm) | Agency | Duration | Comments |
|---|---|---|---|
| 0.1 | AIHA [a] | ERPG- 1<br>1 hour | Maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or perceiving a clearly defined objectionable odor; based on human odor detection. |
| 0.33 | EPA [b] | AEGL 1<br>8 hours | Airborne concentration above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure. |
| 0.36 | EPA [b] | AEGL 1<br>4 hours | |
| 0.51 | EPA [b] | AEGL 1<br>1 hour | |
| 0.6 | EPA [b] | AEGL 1<br>30 minutes | |
| 0.75 | EPA [b] | AEGL 1<br>10 minutes | |
| 10 | ACGIH [c] | TLV-TWA<br>8hr/day, 40hr/week | Occupational exposure |
| 10 | OSHA [d] | 8hr/day, 40hr/week | Occupational exposure |
| 10 | NIOSH [e] | 10 minutes | Recommended exposure time to 10 ppm in the workplace |
| 15 | ACGIH [c] | TVL-STEL<br>Short periods of time | Occupational exposure for short periods of time |
| 17 | EPA [b] | AEGL 2<br>8 hours | Airborne concentration above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. |
| 20 | EPA [b] | AEGL 2<br>4 hours | |
| 20 | OSHA [d] | Ceiling | Ceiling, if no other exceedence of 10 ppm standard |
| 27 | EPA [b] | AEGL 2<br>1 hour | Airborne concentration above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. |
| 30 | AIHA [a] | ERPG-2<br>1 hour | Maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or symptoms which could impair an individual's ability to take productive action; based on animal studies |
| 31 | EPA [b] | AEGL 3<br>8 hours | Airborne concentration above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death. |
| 32 | EPA [b] | AEGL 2<br>30 minutes | Airborne concentration above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. |
| 37 | EPA [b] | AEGL 3<br>4 hours | Airborne concentration above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death. |
| 41 | EPA [b] | AEGL 2<br>10 minutes | Airborne concentration above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. |
| 50 | OSHA [d] | 10 minute ceiling | If no other measurable exposure during 8 hr shift |
| 50 | EPA [b] | AEGL 3<br>1 hour | Airborne concentration above which it is predicted that the general population, including susceptible individuals, could |

| | | | |
|---|---|---|---|
| 59 | EPA [b] | AEGL 3<br>30 minutes | experience life-threatening health effects or death. |
| 76 | EPA [b] | AEGL 3<br>10 minutes | |
| 100 | NIOSH [d] | IDLH | Immediately Dangerous to Life and Health |
| 100 | AIHA [a] | ERPG-3<br>1 hour | Maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects; based on human studies |

[a] Source: EPA, http://www.epa.gov/oppt/aegl/define.htm and http://www.epa.gov/oppt/aegl/results57.htm

[b] Source: AIHA, 2005 American Industrial Hygiene Association, available at http://www.aiha.org/1documents/Committees/ERP-erpglevels.pdf, and EPA Report, 1993 p.III-11, 13.

[c] EPA "Report to Congress on Hydrogen Sulfide Emissions," p.III-10.

[d] Occupational Safety and Health Administration, 29 CFR 1910.1000, available at http://www.osha.gov/dts/chemicalsampling/data/CH_246800.html

[e] Source: NIOSH is a department within the Centers for Disease Control and Prevention. See http://www.cdc.gov/niosh/npg/npgd0337.html for NIOSH's $H_2S$ exposure recommendations.

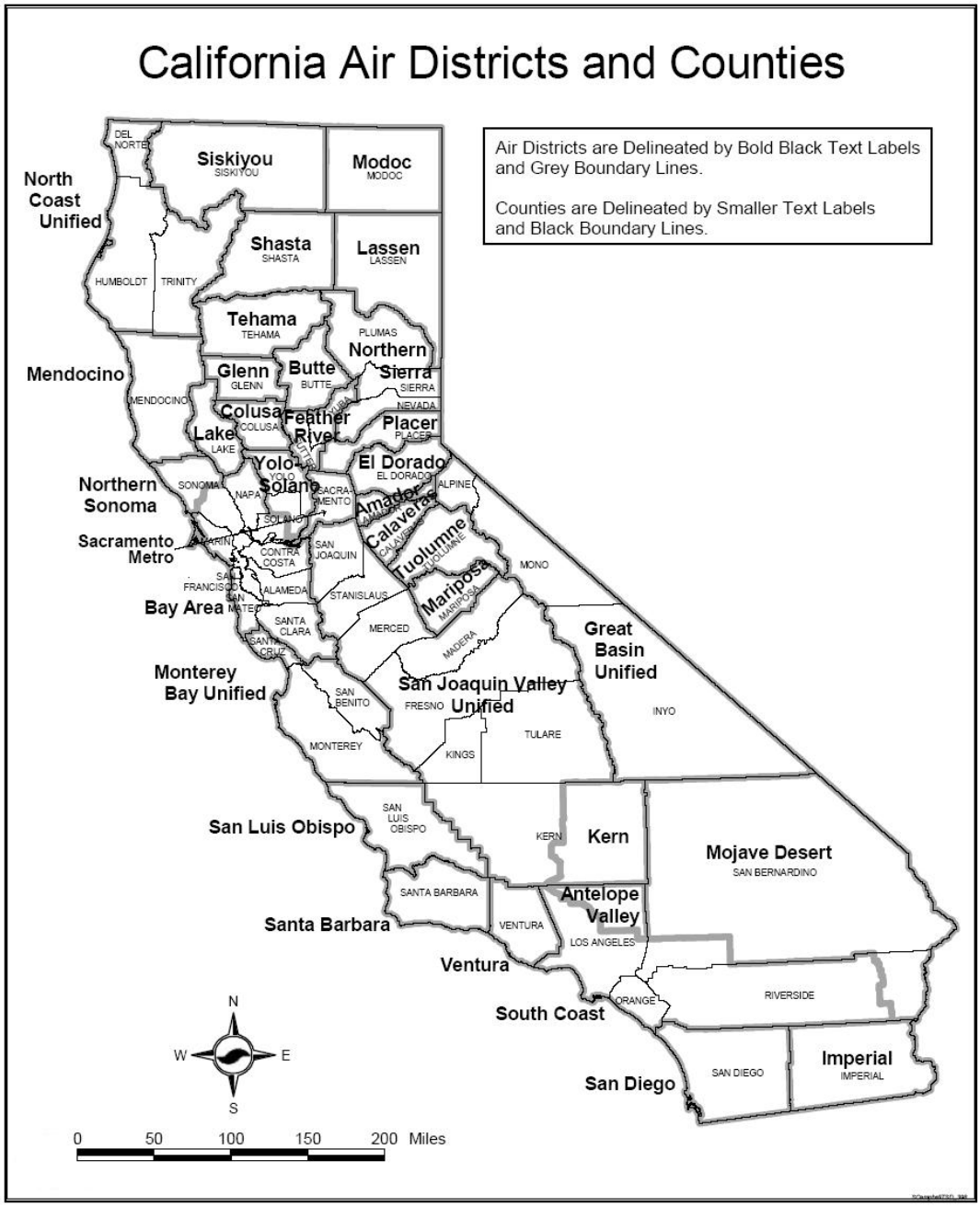
## Appendix B: State Ambient Hydrogen Sulfide Standards

| State | Standard | Duration | Justification | Source |
|---|---|---|---|---|
| Arizona | 180 µg/m3 (0.128 ppm) | 1 hr | AAAQG, health based, on OSHA guidelines | www.azdeq.gov/environ/air/permits/download/ambient.pdf |
| | 110 µg /m3 (0.078ppm) | 24 hr | | |
| California | 0.03 ppm | 1 hr | | California Air Resources Board, Nov 2005: http://www.arb.ca.gov/aqs/aaqs2.pdf |
| Delaware | 0.06 ppm | average concentration not to be exceeded taken over any consecutive 3 minutes | | Regulation 3, Delaware Ambient Air Quality Standards, www.dnrec.state.de.us/air/aqm_page/docs/pdf/reg_3.pdf |
| | 0.03 ppm | average concentration not to be exceeded taken over any consecutive 60 minutes | | |
| Hawaii | 25 ppb | 1 hr | Combination of health and nuisance | Hawaii State Ambient Air Quality Standards, www.hawaii.gov/health/environmental/air/chart.pdf |
| Iowa | 30 ppb | 1-hr daily maximum | "health effects standard" | www.legis.state.ia.us/Rules/2004/Bulletin/IAB040818.pdf |
| Louisiana | 330 ppb | 8-hr average | NIOSH/OSHA safety standard, took 1/42 of their level | Personal Communication, Jim Hazlett, Air Quality Assessment, Louisiana Department of Environmental Quality |
| Massachusetts | 0.65 ppb | 24-hr and annual limit | Based on EPA RfC, Threshold Effects Exposure Limit and Allowable Ambient Limit | Massachusetts Rule 310: Ambient Air Exposure Limits for Chemicals www.mass.gov/dep/air/aallist.pdf |
| Minnesota | 0.05 ppm (70 µg /m3) | 1/2 hr average not to be exceeded over 2 times per year | | Minnesota Pollution Control Agency, State Ambient Air Quality Standards, Chapter 7009.0080 www.revisor.leg.state.mn.us/arule/7009/0080.html |
| | 0.03 ppm (42 µg /m3) | 1/2 hr average not to be exceeded over 2 times in any 5 consecutive days | | |
| Missouri | 0.05 ppm (70 µg /m3) | 1/2 hr average not to be exceeded over 2 times per year | | Missouri Ambient Air Quality Standards CSR 10-6.010, www.sos.mo.gov/adrules/csr/current/10csr/10c10-6a.pdf |
| | 0.03 ppm (42 µg /m3) | 1/2 hr average not to be exceeded over 2 times in any 5 consecutive days | | |
| Montana | 0.05 ppm | hourly average, not to be exceeded more than once per year | health based | Montana Rule 17-8-214 http://deq.mt.gov/dir/legal/Chapters/CH08-02.pdf |
| Nevada | 0.08 ppm | 1-hr average | health based | Nevada Chapter 445B – Air Controls, section 22097, www.leg.state.nv.us/NAC/NAC-445B.html#NAC445BSec22097 |
| New Mexico | 0.010 ppm | 1-hr average not to be exceeded more than once per year | | New Mexico Ambient Air Quality Standards, Title 20, Chapter 2, Part 3 www.nmenv.state.nm.us/aqb/regs/20_2_03nmac_103102.pdf |
| | 0.100 ppm | 1/2 hour average | special for the Pecos-Permian Basin Intrastate Air Quality Control Region | |
| | 0.030 ppm | 1/2 hour average | for within five miles of municipalities in Pecos-Permian Basin that are populated areas (more than 20,000 people) | |

| | | | | |
|---|---|---|---|---|
| New York | 0.01 ppm | 1-hr average | odor and aesthetic | New York Rules and Regulations, Chapter III, Subpart 257-10; www.dec.state.ny.us/website/regs/subpart257_10.html |
| North Dakota | 10 ppm | ceiling, maximum instantaneous concentration not to be exceeded | health based | North Dakota Ambient Air Quality Standards, Chapter 33-15-2 www.legis.nd.gov/information/acdata/html/..%5Cpdf%5C33-15-02.pdf |
| | 0.20 ppm | maximum 1-hr average concentration not to be exceeded more than once per month | | |
| | 0.10 ppm | maximum 24-hr average concentration not to be exceeded more than once per year | | |
| | 0.02 ppm | maximum arithmetic mean concentration averaged over three consecutive months | | |
| Oklahoma | 200 ppb | 24-hr average concentration | | Oklahoma Air Pollution Control Rules, Title 252, Chapter 100-31-7 www.deq.state.ok.us/rules/100.pdf |
| Oregon | 2 µg /m3 (0.3 ppb)* | annual average concentration | based on EPA's RfC, proposed benchmark | Personal Communication, Bruce Hope, Senior Environmental Toxicologist, Oregon Department of Environmental Quality, Air Quality Division. Feb. 10, 2006. |
| Pennsylvania | 0.005 ppm | 24-hr average | | Pennsylvania Article III, Chapter 131, www.pacode.com/secure/data/025/chapter131/025_0131.pdf |
| | 0.1 ppm | 1-hr average | | |
| Texas | 0.08 ppm | 30-min average | if the downwind concentration of hydrogen sulfide affects a property used for residential, business, or commercial purposes | Texas Administrative Code, Title 30 Part 1, Chapter 112, subchapter B; http://info.sos.state.tx.us/pls/pub/readtac$ext.ViewTAC?tac_view=5&ti=30&pt=1&ch=112&sch=B&rl=Y |
| | 0.12 ppm | 30-min average | if the downwind concentration of hydrogen sulfide affects only property used for other than residential, recreational, business, or commercial purposes, such as industrial property and vacant tracts and range lands not normally occupied by people. | |
| Vermont | 33.3 µg /m3 (0.024 ppm) | 24-hr | health based | proposing 1 µg /m3 annual average, to be determined in April; current standard available at http://www.anr.state.vt.us/air/docs/apcregs.pdf |
| Wyoming | 70 µg /m3 (0.05 ppm) | 1/2 hour average not to be exceeded more than 2 times per year | | Wyoming Department of Environmental Quality, Air Quality Division, Ambient Air Quality Standards, Chapter 2: http://deq.state.wy.us/aqd/stnd/Chapter2_2-3-05FINAL_CLEAN.pdf |
| | 40 µg /m3 (0.03 ppm) | 1/2 hour average not to be exceeded more than 2 times in any 5 consecutive days | | |

* Proposed, to be reviewed April 2, 2006

## Appendix C: California Air Districts



## Appendix D: Interview Narratives

*Story 1*

One participant, a 76-year-old rancher, used to own and live on a ranch on flat prairie land in Alberta, Canada. His property was three quarters of a mile straight east and downwind of an oil facility, which included a tank battery where impurities such as sand and salt water were removed from the oil to prepare it for the pipeline. According to government figures which he claims to have obtained, the oil initially contained 8% hydrogen sulfide. This figure increased to 13% after a few years of production. The interviewee believes that $H_2S$ emissions affected people within a radius of up to three miles from the facility.

Venting to the atmosphere from several big tanks in the battery released hydrogen sulfide, as oil added to the tanks stirred up the settled oil, emitting $H_2S$. For the first three years of the facility's operation, there was no flare, and the hydrogen sulfide would accumulate and then be vented.

This interviewee was exposed to hydrogen sulfide many times before he realized what was taking place. He often woke up dizzy, and reports staggering for a half hour before regaining his balance for the rest of the day. Initially, he thought the dizziness was due to high blood pressure, but tests revealed that his blood pressure was normal. During and after every exposure, he experienced angina pains (pain due to lack of oxygen to the heart muscle) while walking even short distances. After moving away, the angina improved and he is able to control it with medication. Nevertheless, he has undergone six by-pass surgeries. Additionally, he experienced pressure inside his head and short term memory loss.

This interviewee experienced health problems as a result of several specific hydrogen sulfide releases from the tank battery. He believes he was exposed to a very high, but unknown, concentration of hydrogen sulfide on one occasion, when he was harvesting crops approximately 500 feet from the oil facility's fence line. He recalls briefly smelling the rotten egg odor, and then not noticing it further. At that time, he experienced a stiffening of the neck and shoulders, and his head 'froze' in the position he had turned it. Now, if he is exposed to hydrogen sulfide, he experiences similar but not as severe symptoms of the head, neck, and shoulders.

On another occasion, when the rotten egg odor characteristic of hydrogen sulfide was especially strong outside the house, he was sick for three days. During this episode, which he attributes to many small exposures over the three days, his balance was disrupted so that he could not stand when he stood up, and he experienced dry heaving. After this episode, he started to be observant and to study the effects of hydrogen sulfide on human health. He used the Internet and visited other people who had problems due to $H_2S$, and found that his symptoms matched hydrogen sulfide exposure.

The interviewee eventually purchased a hydrogen sulfide monitor, and he reports that there were short periods of time when $H_2S$ concentrations were very high. On one occasion, his 17-year-old grandson experienced knockdown while monitoring for hydrogen sulfide on the property. The monitor recorded 200 ppm at that time. His grandson had been exposed at other times while working on the ranch, and had difficulty in school after the knockdown.

I also spoke with the grandson about his knockdown. He experienced dull headaches in the front of his head whenever he was exposed to $H_2S$. He remembers

having a very intense headache right before the incident. His head and neck stiffened, and he lost consciousness. Since this exposure, he has difficulty remembering details and notices his memory getting progressively worse. His balance is not as good as it used to be, and he experiences hot flashes several times per week.

The older interviewee sought medical help on many occasions because of his health problems. Eight hours of testing by an $H_2S$ specialist ruled out Alzheimer's, Parkinson's, and Lou Gehrig's diseases, as well as progressive blindness and deafness. According to this professional's letter, which the interviewee read to me, the diagnosis was of a "75-year-old man who had extensive exposure" and now suffers from chemical encephalopathy (swelling of the brain) due to $H_2S$, small airways obstruction beyond that due to cigarette smoking and attributable to $H_2S$, $SO_2$, and diesel exhaust, and chemical hypersensitivity due to $H_2S$ exposure. His symptoms are a dry cough, shortness of breath, throat irritation, fatigue, lightheadedness, dizziness, insomnia, lack of concentration, and memory loss. The symptoms that improved after moving are throat and eye irritation and balance, but the others persist.

There have also been problems with cattle in the area, including spontaneous abortions and animals' hooves falling off and not healing.

*Story 2*

A 44-year-old woman has suffered many health impacts from exposure to hydrogen sulfide and from an oil refinery, and there are many pump jacks, collection ponds, and tank batteries close to her residence.

The oil refinery transfers its wastewater and production water to the municipal wastewater treatment facility, which also received wastewater from a dairy facility until 2001.[160] Her husband, who was employed at the wastewater treatment facility and had previously worked for an oil company, is now on permanent Social Security Disability Leave because repeated exposure rendered him physically and psychologically unfit to work. According to a safety company monitoring data that she has seen, she reported that concentrations of 10 ppm are commonplace in the street in front of the wastewater treatment plant. A park where children play is across the street from the plant.

She believes that she is exposed to hydrogen sulfide daily or almost daily. The smell of rotten eggs is regularly present in town and at her residence. Sometimes, the odor is very strong for a short time, "a minute or so," and then she no longer detects it. She thinks this is due to concentrations above the odor threshold, which occurs between 50 and 100 ppm.[161] At other times, visitors new to the area could detect an odor while she could not. She thinks her nose is no longer as sensitive to the odor of $H_2S$ due to chronic exposure.

As a result of her and others' complaints, the Center for Disease Control's ATSDR sent personnel to her property to monitor for $H_2S$. Based on the levels they found in the ambient air, ATSDR has plans to follow up with water and soil sampling on the property. Although the monitors registered $H_2S$, the ATSDR took no action because of a lack of health studies attributing adverse health effects to the measured levels. She does not know the exact levels of $H_2S$ on her property.

---

[160] Since then, the diary has secured a discharge permit from the state Department of Environmental Quality to treat its waters on site. The treatment amounts to holding ponds on adjacent farmland that the dairy purchased, and then discharging the water over their property.
[161] Refer to Health Effects Chart.

She also experiences headaches, balance problems, concentrations problems, eye irritation, problems sleeping, and general pain. The headaches are a daily occurrence, but the severity changes. If she leaves town for several days, the headaches stop. Other problems are low muscle strength, trouble walking, problems with memory retention and reading comprehension, and hypersensitivity of the skin. She believes some of her problems are due to sexual contact with her husband, who had been routinely exposed to much higher levels at the workplace, as high as 200 ppm on one occasion, according to a police report of the accident. Specialists have diagnosed her husband with brain damage and physical damage to the neurological and muscular system from exposure to hydrogen sulfide.

Her symptoms prompted her to seek medical help on many occasions. Because of her low muscle strength, one doctor initially diagnosed her with multiple sclerosis. He later changed the diagnosis to chemical encephalopathy along with peripheral neuropathy. Another doctor has diagnosed her with cystic fibrosis, and she is awaiting results from a breast tissue biopsy. She has experienced immune system disorders and her blood tests show abnormalities that no one can explain. Other people in the area have similar blood problems.

*Story 3*

A couple in their mid-40s lived for eleven years in a rural Colorado area of farmland, orchards, trees, and country roads. They have since moved from that location because of the myriad health problems they were experiencing. A natural gas well was situated directly across the street, less than a thousand feet from their residence. There

were other gas wells close to their property, including several on the mountainside nearby, where they could see many being flared. On that mountainside, there was also a big pit containing wastewater.

The property was situated amidst hills and mountains, and it was often very windy. They continually smelled a rotten egg odor from the well across the street, which caused them to complain to the company that was doing the drilling. At first, the company was responsive, shutting the well off for a few days, draining the tanks, and then turning the rig back on. Soon, however, the couple would notice the smell again, complain, and the company would again shut off the well, drain the tanks, then eventually turn them back on. After a year and a half, the company was no longer as responsive to their complaints or as prompt to take action.

There was flaring for many weeks at both gas wells. According to the husband, the company received several citations for illegal flaring. As a result of the their complaints, the company eventually removed one of the tanks. The company told them that they were smelling sour gas and that they had nothing to worry about. In addition to the smell, they were disturbed by the noise from the well. On many occasions, they would leave for the weekend to escape the noise and the odors.

The couple believe that they were continually exposed to hydrogen sulfide for eleven years from the gas well directly across the street and from a well on an adjacent plot. They did not know what they were exposed to until 2005, but based on information they have since gathered and on professional medical assessments, they believe it was hydrogen sulfide all along. They do not know to what concentrations they were exposed.

The couple experienced health problems, which they now attribute to ongoing exposure to hydrogen sulfide. The wife, who spent more time at home, experienced and continues to experience more and worse symptoms than her husband, who would leave the house during the week for work. Specifically, the wife, who had always been healthy before the gas wells started operating near their residence, experienced pains similar to severe rheumatoid arthritis, though tests did not reveal its presence in her body. She nevertheless took medication for nine years, which did not help with her pain. She also experienced blistering of the skin when showering, severe burns on the bottoms of her feet, and her skin was covered in welts. Her entire body was swollen and her throat burned. She spent months sleeping and vomiting. She also suffered from severe sinus headaches, and both husband and wife experienced rectal bleeding, as did some of their neighbors.

After moving, the wife's arthritis symptoms relaxed, but she still sleeps a lot, and undergoes spells of vomiting. Her body experienced symptoms of detoxification after moving. Eventually, her gall bladder was removed, and showed symptoms of poisoning. By this time, she had lost over 50 pounds and was malnourished. Her gall bladder was enlarged to the size of a small pineapple, and had excessive scar tissue, which the doctor attributed to toxicity. She has been experiencing one especially disturbing, and puzzling, behavior, which started after the couple moved away from their old residence near the gas wells. Although she was born and raised in Southern California and English is her native language, she now speaks with a strange, heavy accent, which at times resembles Russian, German, or Swedish. Because of the many persistent heath problems, she is no longer

considered 'insurable' by medical insurance companies, nor is she able to work. She is now on permanent Social Security disability.

In addition to their health problems, the couple believe hydrogen sulfide in the air around their former residence is responsible for causing the metal siding on their house to peel just two years after installation, although it was supposed to last for 50 years. Further, animals on their property experienced health problems. Two baby llamas died from a pneumonia-type illness, and an older llama had severe sinus problems. They put their dogs to sleep because of tumors. They tried to breed miniature poodles, but all the dogs were sterile.

*Story 4*

In October 2004, a couple in their mid-60s retired to a poor rural county in Texas, a known region of sour gas. There are several sour gas wells near their residence. Since March 2005, their property has been surrounded by several wells, all within one mile of their house, with one well across the street and less than a quarter of a mile away. Additionally, an amine gas treating plant where sour gas is sweetened, which also began operating in March 2005, is a half mile from their residence. According to a public record document that the wife received from the state environmental department in response to one of her complaints, "The facility receives sour natural gas and treats the gas with an amine treater to remove $CO_2$ and $H_2S$."[162]

The couple believe they are exposed to hydrogen sulfide from the amine plant and the sour wells that surround their property and that their exposure is ongoing. They have noticed that odors are worse at night. They are convinced that there are hydrogen sulfide

[162] Personal communication, February 27, 2006.

emissions because of the fact that the region is know for sour gas, because the amine plant's purpose is to sweeten sour gas, and because they have read a permit from the state allowing the amine plant to emit up to four pounds of $H_2S$ per hour into the air. They often notice a strong rotten egg odor, as well as other chemical smells, including a sweet smell. (The latter may be due to benzene, or to high levels of $H_2S$, which has been reported to produce a sickening, sweet smell in concentrations above 30 ppm.[163]) The couple acknowledge that there are other pollutants emitted into the air that they do not know about. There has been no monitoring done on their property, and they do not know to what concentrations they are exposed.

Both husband and wife have experienced a host of adverse health effects since the gas wells and the amine plant began operating. Though neither smokes, nor ever has, they are both experiencing a chronic sore throat. Their other symptoms include congestion, coughing, headaches, swollen eyes, insomnia, occasional nosebleeds, and a general lack of energy. The husband's face burns, as do his hands and eyes. The wife has experienced heart palpitations and is now on heart medication, though she was entirely healthy before moving. They have both seen doctors many times because of their problems, including several visits to the emergency room, but neither has been officially diagnosed with hydrogen sulfide exposure. However, they think this is due to the general lack of knowledge about the subject.

On many occasions, they have been awakened by rotten egg fumes that choked, and burned their eyes, nose, and throat, and made them feel dizzy. At these times, they

[163] Snyder, Jack W., MD, PhD. et al. 1995. "Occupational Fatality and Persistent Neurological Sequelae After Mass Exposure to Hydrogen Sulfide." *American Journal of Emergency Medicine*. p. 200.

leave the house and stay in a hotel, and now have a packed suitcase ready in case they need to evacuate in a hurry.

*Story 5*

A New Mexico couple in their late 40s live a quarter to a half mile from a municipal wastewater treatment facility, which has been receiving wastewater from an oil refinery since 1992. The oil refinery itself is four miles from the couple's residence, and there are several oil fields in the vicinity. The refinery has been there since the 1970s, but until 1992, it injected its wastewater into the ground. The wastewater facility used to receive water from a dairy operation as well.

At their residence, the couple are routinely exposed to hydrogen sulfide, which emanates from the water at the treatment facility. The husband used to work at the wastewater treatment facility, so he was exposed to $H_2S$ more frequently, and to higher concentrations than his wife. Accordingly, his symptoms are much more severe. The couple own an H2S monitor, which sounds an alarm at 10 ppm, a daily occurrence inside and outside the house, and while driving in town. A monitor within the gates of the wastewater facility in the past registered 375 ppm, prompting the facility to be temporarily shut down. Additionally, staff from the Centers for Disease Control (CDC) were in the area for six weeks, studying hydrogen sulfide. They placed two monitors on the couple's property, and although the official results are not yet ready, the CDC staff acknowledged that conditions are bad.

The husband's blood tests showed abnormally high levels of H2S, levels so high that the examining physician thought the sample was from a deceased man. Doctors have also confirmed that the wife has been exposed to hydrogen sulfide. The husband's health

has been deteriorating since 1992, when the refinery started discharging wastewater into the municipal wastewater treatment facility. His symptoms include nosebleeds, headaches, burning eyes, throat itching, itching all over his body, severe headaches, and severe rashes. His skin burns when he sweats. His teeth have been damaged, and he has suffered nerve damage and slurred speech. The wife has experienced rapidly deteriorating eyesight, ringing in her ears, memory problems, has had her gall bladder removed, and, since 1995, has trouble with balance, tremors, trouble walking up and down stairs, and severe migraine headaches.

Both have been examined by several $H_2S$ specialists, and both have been diagnosed with chemical encephalopathy. Each time they are tested, the results are worse. The husband is permanently and totally disabled, and is on Social Security disability.

*Story 6*

This interviewee lived on a 640 acre farm in a relatively flat prairie areas with some undulating hills in Alberta, Canada. A natural gas well said to contain one percent hydrogen sulfide was situated about a third of a mile from the residence. There were other gas wells in the vicinity of her property, and gas was piped from these wells to a site about a mile away, where it was flared. Within three miles from the residence, there were at least a dozen natural gas wells, all with hydrogen sulfide content of one percent. There was also a battery three miles away.

Her first serious exposure occurred as a result of flaring during an initial test soon after the closest well to her house was drilled. The flaring created a jet plane-like sound, shaking the house. At this time, she experienced a headache and felt extremely sick. Her

20-year-old son, who was out walking in the field when the incident occurred, experienced knockdown. He staggered into the house, lost his balance, and collapsed. His skin was a greenish gray color when he collapsed. She contacted a regulatory body, which asked the company to stop operations. However, next day the wells were operating again. This flaring continued for a week, continually exposing her and her family to H2S. She smelled the rotten egg odor of hydrogen sulfide, and the company told her that 1 ppm of H2S was blowing to the residence. They continued to smell H2S several times per week. There has been no monitoring to determine the exact concentrations of H2S at her residence.

Within a month of the acute exposure, she was experiencing extreme fatigue, confusion, anxiety, heart symptoms, shaking and tremors, dizziness, headaches, nosebleeds, memory and cognitive impairments. Exposure to H2S or other chemicals would aggravate many of her symptoms. She also has bronchial asthma symptoms though she has never smoked. Her son developed heart arrhythmia, balance problems, and pneumonia three months after the knockdown. He now walks with a cane, experiences extreme headaches, confusion, ongoing heart problems, skin conditions including psoriasis, and burning in his lungs when exposed to chemicals. Both the interviewee and her son have multiple chemical sensitivities. An H2S specialist has diagnosed them with non-recoverable chemical encephalopathy.

Other people in the area have experienced respiratory problems, and there have been many effects on animals, including abortions and cattle's hooves falling off.

*Story 7*

A 27 year-old interviewee lived half a mile from a gas plant, and a little over half a mile from several oil wells with flares. He has since moved to another residence in the same Alabama county, where over 500 oil and gas are active, and he is still exposed to hydrogen sulfide.

He is convinced that he is continually exposed to hydrogen sulfide because of the presence of the rotten egg odor. The results of a monitoring project at his residence confirm the presence of H2S in concentrations of 10 ppb.[164] In addition to his ongoing exposure, the interviewee recalls nearly experiencing knockdown on one occasion while driving by a sour gas plant about five miles from his former residence. The source of that exposure was a flare at the plant, but the concentration of hydrogen sulfide is not known. At that time, he experienced shortness of breath and felt very near unconsciousness.

When he first moved to his previous residence, he started experiencing blurred vision and a loss of energy. His current health problems consist of brain fog, memory impairment, excessive sleepiness, and a lack of energy and strength. He has also experienced diarrhea, blood in his urine, loss of libido, abnormal heart rhythm, and anxiety-like attacks. Sometimes, he experiences severe and protracted involuntary muscle movements in his arms and legs that last up to a day. Exposure to hydrogen sulfide aggravates his existing symptoms. Since moving to the new residence, his heart symptoms have lessened.

He has seen several doctors about his health problems. One doctor diagnosed him with optic nerve damage, and another with chemical encephalopathy. He has tested

[164] Monitoring done by Lisa Sumi, Research Director, Oil and Gas Accountability Project. August 2005, using Jerome 631 H2S monitor. Data used with permission.

negative for a host of diseases, including Parkinson's. Though he is 27, he said he feels 67, and that not a day goes by when he feels normal.

Cats at his old residence were also affected. They experienced vomiting and weight loss, and exhibited sexually confused behavior.

*Story 8:*

This interviewee, a woman in her 50s, is in the process of moving to eastern Texas from her western Colorado home, where she has lived for ten years and worked as an irrigator. There are two natural gas wells about a mile downwind of her residence, and a shut in well across the street. Flaring and open condensate pits were common at these wells.

Although this interviewee experienced symptoms at her residence, her primary exposure to hydrogen sulfide was while she was working in the area, because many wells dot the fields she was irrigating. She started working near the gas wells in March 2005. One well pad was very close to the inlet for the irrigation water. Within a month, she began experiencing burning and swelling in her nasal passages. Several treatments with antibiotics did not clear her symptoms, and her doctor conceded that he did not know what was causing her problems. After this experience, she began wearing a charcoal filter mask.

Her single major exposure, which resulted in a knockdown, occurred one evening when she was getting out of her truck to turn off the irrigation water. She was approximately 50 feet from the well, when she experienced a blinding headache that made her feel like her head would burst. She then started to collapse and black out. She caught the door of her truck and was dangling there for about five minutes. Her headache then

abated and she started to smell the rotten egg odor of hydrogen sulfide, though she did not smell it when the headache started.

Since the knockdown, this interviewee has developed chemical sensitivities, and her doctor has advised her to move. She requires a respirator to be outside, and even with the respirator, she can only be outside for about an hour. She has installed three air scrubbers to purify the air in her house.

She experiences burning around her eyes and on the exposed skin on her face. Her sinuses burn and itch, and she frequently gets nosebleeds. If she is outside for more than an hour, even with the respirator, she develops ulcers on her tongue and in her mouth, and eventually the glands in her neck and armpits swell. If she ignores these symptoms, she gets nauseated and experiences vomiting and explosive diarrhea. She also has nerve inflammation in her legs, and her balance and short term memory are impaired.

The source of hydrogen sulfide, she believes, are fugitive emissions from the wells, and especially from open condensate tanks. A stack flare was also operating within a mile of her house. No tests have been done to confirm the presence of hydrogen sulfide.

Other people in her area have also been affected. One neighbor has been feeling nauseated, while many people smell the odors from the wells and have upper respiratory infections. The interviewee's new mule, which grazes on land near the wells, has experienced hair falling out. A horse also had his mane thin out and experienced diarrhea during flaring. The horse's hooves fell apart and would not heal, so the interviewee had him put down.

# HYDROGEN SULFIDE:
# EVALUATION OF CURRENT CALIFORNIA AIR QUALITY STANDARDS WITH RESPECT TO PROTECTION OF CHILDREN

**James Collins, Ph.D.**
**David Lewis, Ph.D.**
**Air Toxicology and Epidemiology Section**
**California Office of Environmental Health Hazard Assessment**

**Prepared for**
**California Air Resources Board**
**California Office of Environmental Health Hazard Assessment**

**September 1, 2000**

## A. Extended abstract

The current California Ambient Air Quality Standard (CAAQS) for hydrogen sulfide is 0.03 ppm (30 ppb, 42 $\mu g/m^3$) for one hour. The standard was adopted in 1969 and was based on the geometric mean odor threshold measured in adults. The purpose of the standard was to decrease odor annoyance. The standard was reviewed in 1980 and 1984 (CARB, 1984), and was not changed since no new relevant information had emerged. The U.S. EPA presently does not classify hydrogen sulfide as either a criteria air pollutant or a Hazardous Air Pollutant. However, several countries have short-term (usually 30 minute) standards for hydrogen sulfide, as well as long-term (24 hour) standards.

This report focuses on key studies in humans and animals bearing on the health-protectiveness of the CAAQS for hydrogen sulfide. It also includes a discussion of whether significant adverse health effects would reasonably be expected to occur, especially among infants and children, at exposure concentrations below the CAAQS of 30 ppb, based on the findings of published studies. Additional research on odor sensitivity in infants, children, and adults would be useful in evaluating the standard. This would include: (1) testing of the odor threshold for $H_2S$ using the most current methodology among groups of healthy persons of both sexes in different age ranges; (2) odor testing of hydrogen sulfide in adolescents or younger children to determine their odor threshold for $H_2S$; (3) the identification of children hypersensitive to the odor of hydrogen sulfide; and (4) physiologic testing of anosmic (either specifically anosmic to $H_2S$ or totally anosmic) children at the CAAQS to determine if adverse physiological symptoms occur in the absence of odor detection.

## B. Background

The Mulford-Carrell Air Resources Act of 1967 directed the Air Resources Board to divide California into Air Basins and to adopt ambient air quality standards for each basin (Health and Safety Code (H&SC) Section 39606). The existing California state-wide ambient air quality standard (CAAQS) for hydrogen sulfide of 0.03 ppm (30 ppb, 42 $\mu g/m^3$), averaged over a period of 1 hour and not to be equaled or exceeded, protects against nuisance odor ("rotten egg smell") for the general public. The standard was adopted in 1969 and was based on rounding of the geometric mean odor threshold of 0.029 ppm (range = 0.012 – 0.069 ppm; geometric SD = 0.005 ppm) measured in adults (California State Department of Public Health, 1969). The standard was reviewed by the Department of Health Services in 1980 and 1984, and was not changed since no new relevant information had emerged. OEHHA (1999) formally adopted 30 ppb as the acute Reference Exposure Level (REL) for use in evaluating peak off-site concentrations from industrial facilities subject to requirements in H&SC Section 44300 *et seq*. OEHHA (2000) adopted a level of 8 ppb (10 $\mu g/m^3$) as the chronic Reference Exposure Level (cREL) for use in evaluating long term emissions from Hot Spots facilities. The cREL was based on a study demonstrating nasal histological changes in mice.

At the federal level, U.S. EPA does not currently classify hydrogen sulfide as either a criteria air pollutant or a Hazardous Air Pollutant (HAP). U.S. EPA has developed a (chronic) Reference Concentration (RfC) of 0.001 $mg/m^3$ (1 $\mu g/m^3$) for hydrogen sulfide (USEPA, 1999). The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

There are no international standards for $H_2S$. Many countries have "short-term" (usually 30 minute) standards, which range from 6 to 210 ppb (WHO, 1981). The World Health Organization (WHO) recommends that, in order to avoid substantial complaints about odor

annoyance among the exposed population, hydrogen sulfide concentrations should not be allowed to exceed 0.005 ppm (5 ppb; 7 $\mu g/m^3$), with a 30-minute averaging time (WHO, 1981; National Research Council, 1979; Lindvall, 1970). A very short-lived, peak concentration could also be annoying. Rule 2 of Regulation 9 of the Bay Area Air Quality Management District (BAAQMD) specifies that ambient ground level $H_2S$ concentrations may not exceed 60 ppb averaged over 3 consecutive minutes. Regulating at averaging times less than 30 – 60 minutes may be difficult. Many countries have "long-term" (24 hour) standards (WHO, 1981).

NRC (1979), WHO (1981), Beauchamp *et al*. (1984), Reiffenstein *et al*. (1992), and ATSDR (1999) have published reviews of the health effects of hydrogen sulfide.

## C. Principal sources/Exposure assessment

Hydrogen sulfide ($H_2S$) is used as a reagent and as an intermediate in the preparation of other reduced sulfur compounds (HSDB, 1999). It is also a by-product of desulfurization processes in the oil and gas industries and rayon production, sewage treatment, and leather tanning (Ammann, 1986). Geothermal power plants, petroleum production and refining, and sewer gas are specific sources of hydrogen sulfide in California. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Information and Assessment Act in California (H&SC Sec. 44300 *et seq*.), based on the most recent inventory, were estimated to be 5,688,172 pounds of hydrogen sulfide (CARB, 1999).

A specific concern in California has been schools located near workplaces emitting toxic substances. For example, the Hillcrest Elementary School in Rodeo (Contra Costa County; part of the BAAQMD) is adjacent to an oil refinery which, on occasion, has emitted enough malodorous sulfur compounds (including $H_2S$) for the school to close its doors and for the teachers and children to "shelter-in-place." Thus the school district has planned to relocate the school (West County Times, November 23, 1999). These compounds have also affected other schools in the area.

Hydrogen sulfide is produced endogenously in mammalian tissues from L-cysteine, mainly by two pyridoxal-5'-phosphate-dependent enzymes, cystathionine beta-synthetase and cystathionine gamma-lyase (Hosoki *et al*., 1997). Abe and Kimura (1996) suggested that hydrogen sulfide may be an endogenous neuromodulator in the hippocampus based on the high level of cystathionine beta-synthetase in the hippocampus and on experimental effects of activators and inhibitors of the enzyme.

## D. Key studies of acute and chronic health impacts

### D.1. Toxicity to Humans

**D.1.1. Adults**. Hydrogen sulfide is an extremely hazardous gas (ACGIH, 1991). Exposure to high concentrations of hydrogen sulfide is reported to be the most common cause of sudden death in the workplace (NIOSH, 1977). Estimates of the mortality resulting from acute hydrogen sulfide intoxication include 2.8% (Arnold *et al*., 1985) and 6% (WHO, 1981). While severe intoxication is especially of concern when exposure occurs in confined spaces, an accidental release of hydrogen sulfide into the ambient air surrounding industrial facilities can cause very serious effects. As a result of an accidental release of hydrogen sulfide due to a malfunctioning flare at an oilfield at Poza Rica, Mexico in 1950, 320 people were hospitalized and 22 died (WHO, 1981).

Most information on $H_2S$ toxicity comes from studies that used levels of $H_2S$ orders of magnitude above the standard of 0.03 ppm. Hazardtext (1994) reported an inhalation $LC_{Lo}$ of 600 and 800 ppm (840 and 1,120 mg/m³) for 30 and 5 minutes, respectively. A lethal exposure was documented for a worker exposed to approximately 600 ppm $H_2S$ for 5 to 15 minutes (Simson and Simpson, 1971). Inhalation of 1,000 ppm (1,400 mg/m³) is reported to cause immediate respiratory arrest (ACGIH, 1991). Concentrations greater than 200 ppm (280 mg/m³) $H_2S$ are reported to cause direct irritant effects on exposed surfaces and can cause pulmonary edema following longer exposures (Spiers and Finnegan, 1986). The mechanism of $H_2S$

toxicity, cellular hypoxia caused by inhibition of cytochrome oxidase, is similar to that for cyanide. Toxicity can be treated by induction of methemoglobin or by therapy with hyperbaric oxygen (Elovaara *et al*., 1978; Hsu *et al*., 1987).

At concentrations exceeding 50 ppm (70 mg/m$^3$) $H_2S$, olfactory fatigue prevents detection of $H_2S$ odor. Exposure to 100-150 ppm (140-210 mg/m$^3$) for several hours causes local irritation (Haggard, 1925). Exposure to 50 ppm for 1 hour causes conjunctivitis with ocular pain, lacrimation, and photophobia; this can progress to keratoconjunctivitis and vesiculation of the corneal epithelium (ACGIH, 1991).

Bhambhani and Singh (1985) reported that exposure of 42 individuals to 2.5 to 5 ppm (3.5 to 7 mg/m$^3$) $H_2S$ caused coughing and throat irritation after 15 minutes. Bhambhani and Singh (1991) showed that 16 healthy adult male subjects (25.2±5.5 years old) exposed to 5 ppm (7 mg/m$^3$) $H_2S$ under conditions of moderate exercise exhibited impaired lactate and oxygen uptake in the blood. Subsequently Bhambani *et al*. (1994) compared the effects of inhaling 5 ppm $H_2S$ on physiological and hematological responses during exercise. Subjects were 13 men (mean±SD for age, height, and weight = 24.7±4.6 y, 173±6.6 cm, and 73.1±8.1 kg, respectively) and 12 women (mean±SD = 22.0±2.1 y, 165±8.2 cm, and 63.4±8.6 kg, respectively). Subjects completed two 30-minute exercise tests on a cycle ergometer at 50% of their predetermined maximal aerobic power, while breathing either air or 5 ppm $H_2S$. There were no significant differences between the two exposures for metabolic (oxygen uptake, carbon dioxide production, respiratory exchange ratio), cardiovascular (heart rate, blood pressure, rate pressure product), arterial blood (oxygen and carbon dioxide tensions, pH), and perceptual (rating of perceived exertion) responses. No one reported adverse health effects following $H_2S$ exposure. The authors believe that healthy adults can safely perform moderate intensity work in environments containing 5 ppm $H_2S$.

Bhambhani *et al*. (1996) examined the acute effects of "oral" inhalation of 10-ppm $H_2S$, the occupational exposure limit, on lung physiology as measured by pulmonary function in nine men and ten women. The volunteers inhaled medical air or 10 ppm $H_2S$ through the mouth for 15 minutes each during cycle exercise at 50% of their maximal aerobic power. Routine pulmonary function tests (FVC, $FEV_1$, $FEV_1$/FVC, PEFR, maximal ventilation volume, and $DL_{CO}$) were administered at rest and immediately after the two exposure conditions. There were no significant changes in any of the variables derived from the flow volume loop, maximum ventilation volume, and diffusion capacity of the lung for carbon monoxide ($DL_{CO}$) in both genders. No subject experienced any sign or symptom as a result of $H_2S$. The authors concluded that inhalation of 10 ppm $H_2S$ through the mouth at an elevated metabolic and ventilation rate does not significantly alter pulmonary function in healthy people.

Jappinen *et al*. (1990) exposed ten adult asthmatic volunteers to 2 ppm $H_2S$ for 30 minutes and tested pulmonary function. All subjects reported detecting "very unpleasant" odor but "rapidly became accustomed to it." Three subjects reported headache following exposure. No significant changes in mean FVC or $FEV_1$ were reported. Although individual values for specific airway resistance ($SR_{aw}$) were not reported, the difference following exposure ranged from –5.95% to +137.78%. The decrease in specific airway conductance, $SG_{aw}$, ranged from –57.7% to +28.9%. The increase in mean $SR_{aw}$ and the decrease in mean $SG_{aw}$ were not statistically significant for the entire group. However, markedly (>30%) increased airway resistance and decreased airway conductance were noted in two of the ten asthmatic subjects at 2 ppm, which indicated bronchial obstruction and may be clinically important. Two ppm is 67 times the CAAQS of 0.03 ppm.

Hydrogen sulfide is noted for its strong and offensive odor. The existing CAAQS of 0.03 ppm (30 ppb, 42 $\mu g/m^3$) for 1 hour is based on rounding the geometric mean odor detection threshold of 0.029 ppm (range = 0.012 – 0.069 ppm; GSD = 0.005 ppm). The threshold was

determined for a panel of 16 presumably healthy adults (California State Department of Public Health, 1969). No information on the sex or age of the panel members has been located. Amoore (1985) reviewed 26 studies, published between 1848 and 1979, all of which reported average odor detection thresholds for $H_2S$. The 26 studies seem to be mainly controlled exposures and used various measurement methods. They included (1) at least two studies using only one subject, (2) a study of a panel of 35 people testing odors in natural gas in Southern California, and (3) another study of 852 untrained young adults (age range = 17.5 – 22.4 years) tested at county and state fairs in the Northwest. The average odor detection threshold in the 26 studies ranged from 0.00007 to 1.4 ppm $H_2S$. The geometric mean of the 26 studies was 0.008 ppm (8 ppb), approximately one-fourth the value determined by the Department of Public Health and lower than the lowest individual threshold of 12 ppb measured in the California panel. Surprisingly the Department of Public Health panel study was <u>not</u> one of the 26 studies used by Amoore and was not even mentioned in his 1985 report to the ARB.

Venstrom and Amoore (1968) reported that, in general, olfactory sensitivities decrease by a factor of 2 for each 22 years of age above age 20. The conclusion was based on a study of 18 odorants in 97 government laboratory workers, ages 20 through 70. Hydrogen sulfide was not tested. The geometric mean odor threshold of 8 ppb for $H_2S$ from the 26 studies is based on an average age of 40 (possibly assumed to be the age of an average adult). Amoore (1985) estimated that an 18-year-old person would have a threshold of 4 ppb $H_2S$, while a 62-year-old person was predicted to have a threshold of 16 ppb. Amoore also stated that there was no noticeable trend of odor sensitivity between young adults and children down to 5 years but did not present specific data to support the statement.

Concentrations, which substantially exceed the odor threshold for, result in the annoying and discomforting physiological symptoms of headache or nausea (Amoore, 1985; Reynolds and Kauper 1984). The perceived intensity of the odor of $H_2S$ depends on the longevity of the concentration, and the intensity increases 20% for each doubling of the concentration (Amoore,

1985). Several studies have been conducted to establish the ratio of discomforting annoyance threshold to detection threshold for unpleasant odors (Winkler, 1975; Winneke and Kastka, 1977; Hellman and Small, 1974; Adams *et al.,* 1968; and NCASI, 1971). The geometric mean for these studies is 5; therefore an unpleasant odor should result in annoying discomfort when it reaches an average concentration of 5 times its detection threshold. (Two studies that tested only $H_2S$ had a geometric mean of 4.) Applying the 5-fold multiplier to the mean detectable level of 8 ppb results in a mean annoyance threshold of 40 ppb. Amoore (1985) estimates that at 30 ppb, the CAAQS, $H_2S$ would be detectable by 83% of the population and would be discomforting to 40% of the population (Table 1). These "theoretical" estimates have been substantiated by odor complaints and reports of nausea and headache (Reynolds and Kauper 1984) at 30 ppb $H_2S$ exposures from geyser emissions.

In order to avoid substantial complaints about odor annoyance among the exposed population, the World Health Organization (WHO) recommends that hydrogen sulfide concentrations should not exceed 0.005 ppm (5 ppb; 7 $\mu g/m^3$), with a 30-minute averaging time (WHO, 1981; National Research Council, 1979; Lindvall, 1970). The WHO task group believed that 5 ppb averaged over 30 minutes "should not produce odour nuisance in most situations."

**Table 1. Predicted effects of exposure to ambient $H_2S$. (Adapted from Amoore, 1985)**

| $H_2S$ (ppb) | % able to detect odor[a] | Perceived odor intensity[b] (ratio) | Median odor units[c] | % annoyed by odor[d] |
|---|---|---|---|---|
| 200 | 99 | 2.31 | 25 | 88 |
| 100 | 96 | 1.93 | 12 | 75 |
| 50 | 91 | 1.61 | 6.2 | 56 |
| 40 | 88 | 1.52 | 5.0 | 50 |
| 35 | 87 | 1.47 | 4.4 | 47 |
| 30 (CAAQS) | 83 | 1.41 | 3.7 | 40 |
| 25 | 80 | 1.34 | 3.1 | 37 |
| 20 | 74 | 1.27 | 2.5 | 31 |
| 15 | 69 | 1.18 | 1.9 | 22 |
| 10 | 56 | 1.06 | 1.2 | 17 |
| 8 | 50 | 1.00 | 1.00 | 11 |
| 6 | 42 | 0.93 | 0.75 | 8 |
| 4 | 30 | 0.83 | 0.50 | 5 |
| 2 | 14 | 0.70 | 0.25 | 2 |
| 1 | 6 | 0.58 | 0.12 | 1 |
| 0.5 | 2 | 0.49 | 0.06 | 0 |

[a]Based on mean odor detection threshold of 8.0 ppb and SD±2.0 binary steps
[b] Based on intensity exponent of 0.26 (Lindvall, 1974).
[c]$H_2S$ concentration divided by mean odor detection threshold of 8 ppb.
[d] Based on assumption that mean annoyance threshold is 5x the mean odor detection threshold, and SD±2.0 binary steps.

Kilburn and Warshaw (1995) investigated whether people exposed to sulfide gases, including $H_2S$, as a result of working at or living downwind from the processing of "sour" crude oil demonstrated persistent neurobehavioral dysfunction. They studied 13 former workers and 22 neighbors of a California coastal oil refinery who complained of headaches, nausea, vomiting, depression, personality changes, nosebleeds, and breathing difficulties. Neurobehavioral functions and a profile of mood states were compared to 32 controls matched for age and educational level. The exposed subjects' mean values were statistically significantly different (abnormal) compared to controls for several tests (two-choice reaction time; balance (as speed of sway); color discrimination; digit symbol; trail-making A and B; immediate recall of a story). Their profile of mood states (POMS) scores were much higher than those of controls. Test scores for anger, confusion, depression, tension-anxiety, and fatigue were significantly

elevated and nearly identical in both exposed residents and former workers, while the scores for controls equaled normal values from other published studies. Visual recall was significantly impaired in neighbors, but not in the former workers. Limited off-site air monitoring (one week) in the neighborhood found average levels of 10 ppb $H_2S$ (with peaks of 100 ppb), 4 ppb dimethylsulfide, and 2 ppb mercaptans. On-site levels were much higher. The authors concluded that neurophysiological abnormalities were associated with exposure to reduced sulfur gases, including $H_2S$ from crude oil desulfurization.

**D.1.2. Children.** In a case report Gaitonde *et al*. (1987) described subacute encephalopathy, ataxia, and choreoathetoid (jerky, involuntary) responses in a 20-month-old child with long term (approximately one year) exposure to hydrogen sulfide from a coal mine. Levels of up to at least 0.6 ppm (600 ppb) were measured and levels were possibly higher before measurements started. The abnormalities resolved after the emission source ceased operation.

As part of the South Karelia Air Pollution Study in Finland (Jaakkola *et al*., 1990), Marttila *et al*. (1994) assessed the role of long-term exposure to ambient air malodorous sulfur compounds released from pulp mills as a determinant of eye and respiratory symptoms and headache in children. The parents of 134 children living in severely polluted (n = 42), moderately polluted (n = 62), and rural, non-polluted (n = 30) communities responded to a cross-sectional questionnaire (response rate = 83%). In the severely polluted area, the annual mean concentrations of hydrogen sulfide and methyl mercaptan ($H_3CSH$) were estimated to be 8 $\mu g/m^3$ (6 ppb) and 2 - 5 $\mu g/m^3$ (1.4 – 3.6 ppb), respectively. The highest daily average concentrations were 100 $\mu g/m^3$ (71 ppb) and 50 $\mu g/m^3$ (36 ppb), respectively. The adjusted odds ratios (OR) for symptoms experienced during the previous 4 weeks and 12 months in the severely versus the non-polluted community were estimated in logistic regression analysis controlling for age and gender. The risks of nasal symptoms, cough, eye symptoms, and

headache were increased in the severely polluted community, but did not reach statistical significance (Table 2). In addition, OEHHA staff noted that the highest percentages of children with symptoms were in the moderately polluted community, not in the severely polluted community. The authors concluded that exposure to malodorous sulfur compounds may affect the health of children. The odor threshold for methyl mercaptan of 1.6 ppb (Amoore and Hautala, 1983) indicates that it also likely contributed to the odor and probably the symptoms.

**Table 2. Symptoms Reported in Marttila *et al*. (1994)**

| *Symptom* | *Time* | *Odds Ratio* | *95% CI* | *Time* | *Odds ratio* | *95%CI* |
|---|---|---|---|---|---|---|
| nasal symptoms | 4 weeks | 1.40 | 0.59-3.31 | 12 months | 2.47 | 0.93-6.53 |
| cough | 4 weeks | 1.83 | 0.75-4.45 | 12 months | 2.28 | 0.95-5.47 |
| eye symptoms | NR | NR | NR | 12 months | 1.15 | 0.43-3.05 |
| headache | NR | 1.02 | 0.36-2.94 | 12 months | 1.77 | 0.69-4.54 |

NR = not reported

Studies of controlled exposures in children to study $H_2S$ odor detection have not been located. A recent report studying children concluded that children aged 8 to 14 years have equivalent odor sensitivity to young adults (Cain *et al*., 1995), although children lack knowledge to identify specific odors by name. Koelega (1994) found that prepubescent children (58 nine-year-olds) were inferior in their detection of 4 of 5 odors compared to 15-year-olds (n = 58) and 20-year-olds (n = 112). Schmidt and Beauchamp (1988) have even tested 3-year-olds (n = 16) for sensitivity to noxious chemicals, such as butyric acid and pyridine.

In March-April 1983, 949 cases (including 727 in adolescent females) of acute non-fatal illness consisting of headache, dizziness, blurred vision, abdominal pain, myalgia, and fainting occurred at schools on the West Bank. However, physical examinations and biochemical tests were normal. There was no common exposure to food, drink, or agricultural chemicals among those affected. No toxins were consistently present in patients' blood or urine. The only environmental toxicant detected was $H_2S$ gas in low concentrations (40 ppb) in a schoolroom at

the site of the first outbreak (from a faulty latrine in the schoolyard). The illness was deemed to be psychogenic and possibly triggered by the smell of $H_2S$ (Landrigan and Miller , 1983; Modan *et al*., 1983).

**D.1.3. Development**. Xu *et al*. (1998) conducted a retrospective epidemiological study in a large petrochemical complex in Beijing, China in order to assess the possible association between petrochemical exposure and spontaneous abortion. The facility consisted of 17 major production plants divided into separate workshops, which allowed for the assessment of exposure to specific chemicals. Married women (n = 2,853), who were 20-44 years of age, had never smoked, and who reported at least one pregnancy during employment at the plant, participated in the study. According to their employment record, about 57% of these workers reported occupational exposure to petrochemicals during the first trimester of their pregnancy. There was a significantly increased risk of spontaneous abortion for women working in all of the production plants with frequent exposure to petrochemicals compared with those working in non-chemical plants. Also, when a comparison was made between exposed and non-exposed groups within each plant, exposure to petrochemicals was consistently associated with an increased risk of spontaneous abortion (overall odds ratio (OR) = 2.7 (95% confidence interval (CI) = 1.8 to 3.9) after adjusting for potential confounding factors). Using exposure information obtained from interview responses for (self-reported) exposures, the estimated OR for spontaneous abortions was 2.9 (95% CI = 2.0 to 4.0). When the analysis was repeated by excluding 452 women who provided inconsistent reports between recalled exposure and work history, a comparable risk of spontaneous abortion (OR 2.9; 95% CI = 2.0 to 4.4) was found. In analyses for exposure to specific chemicals, an increased risk of spontaneous abortion was found with exposure to most chemicals. There were 106 women (3.7% of the study population) exposed only to hydrogen sulfide; the results for $H_2S$ (OR 2.3; 95% CI = 1.2 to 4.4) were statistically significant. Unfortunately $H_2S$ exposure concentrations were not reported.

## D.2. Effects of Animal Exposure

**D.2.1. Adult/mature animals**. A median lethal concentration ($LC_{50}$) in rats exposed to $H_2S$ for 4 hours was estimated as 440 ppm (616 mg/m³) (Tansy *et al*., 1981). An inhalation $LC_{Lo}$ of 444 ppm for an unspecified duration is reported in rats, and a lethal concentration of 673 ppm (942 mg/m³) for 1 hour is reported in mice (RTECS, 1994). In another study, mortality was significantly higher for male rats (30%), compared to females (20%), over a range of exposure times and concentrations (Prior *et al*., 1988). A concentration of 1,000 ppm (1,400 mg/m³) caused respiratory arrest and death in dogs after 15-20 minutes (Haggard and Henderson, 1922). Inhalation of 100 ppm (140 mg/m³) for 2 hours resulted in altered leucine incorporation into brain proteins in mice (Elovaara *et al*., 1978). Kosmider *et al*. (1967) reported abnormal electrocardiograms in rabbits exposed to 100 mg/m³ (71 ppm) $H_2S$ for 1.5 hours.

Khan *et al*. (1990) exposed groups of 12 male Fischer 344 rats to 0, 10, 50, 200, 400, or 500-700 ppm hydrogen sulfide for 4 hours. Four rats from each group were euthanized at 1, 24, or 48 hours post-exposure. The activity of cytochrome c oxidase in lung mitochondria, a primary molecular target of $H_2S$, was significantly ($p<0.05$) decreased at 50 ppm (15%), 200 ppm (43%), and 400 ppm (68%) at 1-hour post-exposure compared to controls. A NOAEL of 10 ppm for inhibition of cytochrome c oxidase was identified in this study.

Fischer and Sprague-Dawley rats (15 per group) were exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m$^3$, respectively) $H_2S$ for 6 hours/day, 5 days/week for 90 days (CIIT, 1983a,b). Measurements of neurological and hematological function revealed no abnormalities due to $H_2S$ exposure. Histological examination of the nasal turbinates also revealed no significant exposure-related changes. A significant decrease in body weight was observed in both strains of rats exposed to 80 ppm (112 mg/m$^3$).

In a companion study, the CIIT conducted a 90-day inhalation study in mice (10 or 12 mice per group) exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m$^3$, respectively) $H_2S$ for 6 hours/day, 5 days/week (CIIT, 1983c). Neurological function was measured by tests

for posture, gait, facial muscle tone, and reflexes. Ophthalmologic and hematologic examinations were also performed, and a detailed necropsy was included at the end of the experiment. The only exposure-related histological lesion was inflammation of the nasal mucosa of the anterior segment of the noses of mice exposed to 80 ppm (112 mg/m$^3$) $H_2S$. Weight loss was also observed in the mice exposed to 80 ppm. Neurological and hematological tests revealed no abnormalities. The 30.5 ppm (42.5 mg/m$^3$) level was considered to be a NOAEL for histological changes in the nasal mucosa. (Different adjustments were made to this NOAEL by U. S. EPA to calculate the RfC of 1 μg/m$^3$ and by OEHHA to calculate the chronic REL of 10 μg/m$^3$ (8 ppb).)

Hydrogen sulfide (0, 10, 30, or 80 ppm) was administered via inhalation (6 h/d, 7 d/wk) to 10-week-old male CD rats (n = 12/group) for 10 weeks (Brenneman *et al*., 2000). Histological evaluation revealed that rats exposed to 30 or 80 ppm had significant increases in lesions of the olfactory mucosa but not other tissues. Multifocal, rostrocaudally-distributed olfactory neuron loss and basal cell hyperplasia were seen. The dorsal medial meatus and the dorsal and medial portions of the ethmoid recess were affected. The lowest dose (10 ppm) was considered a no observed adverse effect level for olfactory lesions.

Fischer F344 rats inhaled 0, 1, 10, or 100 ppm hydrogen sulfide for 8 hours/day for 5 weeks (Hulbert *et al*, 1989). No effects were noted on baseline measurements of airway resistance, dynamic compliance, tidal volume, minute volume, or heart rate. Two findings were noted more frequently in exposed rats: (1) proliferation of ciliated cells in the tracheal and bronchiolar epithelium, and (2) lymphocyte infiltration of the bronchial submucosa. Some exposed animals responded similarly to controls to aerosol methacholine challenge, whereas a subgroup of exposed rats were hyperreactive to concentrations as low as 1 ppm $H_2S$.

Male rats were exposed to 0, 10, 200, or 400 ppm $H_2S$ for 4 hours (Lopez *et al.*, 1987). Samples of bronchoalveolar and nasal lavage fluid contained increased inflammatory cells,

protein, and lactate dehydrogenase in rats treated with 400 ppm. Later Lopez and associates (1988) showed that exposure to 83 ppm (116 mg/m$^3$) for 4 hours resulted in mild perivascular edema.

**D.2.2. Developing animals.** Saillenfait *et al.* (1989) investigated the developmental toxicity of $H_2S$ in rats. Rats were exposed 6 hours/day on days 6 through 20 of gestation to 100 ppm hydrogen sulfide. No maternal toxicity or developmental defects were observed.

Hayden *et al*. (1990) exposed gravid Sprague-Dawley rat dams continuously to 0, 20, 50, and 75 ppm $H_2S$ from day 6 of gestation until day 21 postpartum. The animals demonstrated normal reproductive parameters until parturition, when delivery time was extended in a dose-dependent manner (with a maximum increase of 42% at 75 ppm). Pups exposed in utero and neonatally to day 21 postpartum developed with a subtle decrease in time of ear detachment and hair development, but with no other observed change in growth and development through day 21 postpartum.

Hannah and Roth (1991) analyzed the dendritic fields of developing Purkinje cells in rat cerebellum to determine the effects of chronic exposure to low concentrations of $H_2S$ during perinatal development. Treatment of timed-pregnant female Sprague Dawley rats with 20 and 50 ppm $H_2S$ for 7 hours per day from day 5 after mating until day 21 after birth produced severe alterations in the architecture and growth characteristics of the dendritic fields of the Purkinje cells. The architectural modifications included longer branches, an increase in the vertex path length, and variations in the number of branches in particular areas of the dendritic field. The treated cells also exhibited a nonsymmetrical growth pattern at a time when random terminal branching is normally occurring. Thus, developing neurons exposed to $H_2S$ may be at risk of severe deficits. However, the lower level of 20 ppm for 7 hours is nearly 2 orders of magnitude above the present one-hour standard.

Dorman *et al*. (2000) examined the effect of perinatal exposure of $H_2S$ on pregnancy outcomes, offspring development, and offspring behavior in rats. Male and female Sprague-

Dawley rats (12 rats/sex/concentration) were exposed to 0, 10, 30, or 80 ppm $H_2S$ 6 h/day, 7 days/week for 2 weeks prior to breeding. Exposures continued during a 2-week mating period and then from Gestation Day (GD) 0 through GD 19. Exposure of rat dams and their pups (eight rats/litter after culling) resumed between postnatal day (PND) 5 and 18. Adult males were exposed for 70 consecutive days. Offspring were evaluated using motor activity (assessed on PND 13, 17, 21, and 60±2), passive avoidance (PND 22±1 and 62±3), functional observation battery (FOB) (PND 60±2), acoustic startle response (PND 21 and 62±3), and neuropathology (PND 23±2 and 61±2). No deaths occurred and no adverse physical signs were seen in $F_0$ males or females. There were no statistically significant effects on the reproductive performance of the $F_0$ rats as assessed by the number of females with live pups, litter size, average length of gestation, and the average number of implants per pregnant female. Exposure to $H_2S$ did not affect pup growth, development, or performance on any behavioral test. The authors conclude that $H_2S$ is neither a reproductive toxicant nor a behavioral developmental neurotoxicant in the rat at occupationally relevant exposure concentrations (i.e., at 10 ppm, the current occupational daily average exposure limits - TLV and PEL; however, the ACGIH is considering lowering the TLV to 5 ppm). The lowest level tested (10 ppm) is more than 300-fold higher than the CAAQS of 0.030 ppm.

## E. Interactions between hydrogen sulfide and other pollutants

Ethanol can potentiate the effects of $H_2S$ by shortening the mean time-to-unconsciousness in mice exposed to 800 ppm (1,120 mg/m³) $H_2S$ (Beck *et al*., 1979).

Endogenous hydrogen sulfide may regulate smooth muscle tone in synergy with nitric oxide (Hosoki *et al*., 1997).

Hydrogen sulfide is often accompanied by other malodorous sulfur compounds, such as methyl mercaptan, dimethyl sulfide, and dimethyl disulfide. Some of these have odor thresholds

lower than that of hydrogen sulfide. The complex mixture is often referred to as TRS (total reduced sulfur).

Lindvall (1977) reported that the perceived odor strength of $H_2S$ is increased by the simultaneous presence of 600 ppb nitric oxide (600 ppb nitric acid is imperceptible by itself).

## F. Conclusions

The current standard of 0.03 ppm (30 ppb) hydrogen sulfide for one hour based on odor is well below NOAEL levels from animal experiments where exposure lasted weeks to months, including the period of intrauterine development. However, it is greater than OEHHA's chronic Reference Exposure Level (REL) of 8 ppb, which is based on histological changes in the nasal area of mice. (The chronic REL is compared to the annual average $H_2S$ concentration.) Ideally neither of these two benchmark levels should be exceeded by the properly averaged concentration.

Additional research might help reduce uncertainties regarding the impacts of hydrogen sulfide on the health of infants and children. This would include:

a. Odor testing of hydrogen sulfide in adolescents or younger children, if ethically permissible, to determine their odor threshold. Current data on odor detection in children are not consistent. Data on $H_2S$ odor detection in children under controlled exposure are lacking.

b. The identification of children hypersensitive to the odor of hydrogen sulfide. While the odor from very low level $H_2S$ would not itself threaten their physical health, the odor might be alarming to hypersensitive children. Psychosomatic complaints might be more confusing to children than to adults.

c. Physiologic testing of anosmic (either specifically anosmic to $H_2S$ or totally anosmic) children at the CAAQS would be useful in determining whether if adverse physiological symptoms occur in the absence of odor detection.

d. Testing of the odor threshold for $H_2S$ using the most current methodology among groups of healthy persons of both sexes in different age ranges. Data from such testing would likely be an improvement over the use of either the mean of 16 people (California Department of Public Health, 1969) or the mean from 26 studies, conducted over a period of 130 years, which found thresholds spanning a 20,000 fold range, from 0.07 ppb to 1400 ppb (Amoore, 1985). (If the highest and lowest values of the range in Amoore (1985) are dropped as outliers - Amoore (1985) stated that these two studies seemed to involve only one subject - the range would be 0.43 ppb to 190 ppb, a 440-fold range).

e. Further research is needed on the topic of when odor is an adverse health effect and how much consideration should be given to psychosomatic complaints accompanying odor annoyance (Dalton *et al*., 1997; ATS, 2000). A recent American Thoracic Society position paper titled “What Constitutes an Adverse Health Effect of Air Pollution?” (ATS, 2000) indicates that air pollution exposures, which interfere with the quality of life, can be considered adverse. This suggests that, for the purpose of setting a standard, odor-related annoyance should be considered adverse, even if nausea or headache or other symptoms are not present.

## G. References


Abe K, Kimura H. 1996. The possible role of hydrogen sulfide as an endogenous neuromodulator. J. Neurosci. 16(3):1066-1071.

Adams DF, Young FA, Lahr RA. 1968. Evaluation of odor perception threshold test facility. TAPPI 51(13):62A-67A.

ATSDR. 1999. The Agency for Toxic Substances and Disease Registry. Hydrogen sulfide. Atlanta: ATSDR.

(ACGIH) American Conference of Governmental Industrial Hygienists. Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Vol II. Cincinnati: ACGIH; 1991. p. 786-788.

ATS. 2000. American Thoracic Society. What constitutes an adverse health effect of air pollution? Am. J. Respir. Crit. Care Med. 161:655-673.

Ammann HM. 1986. A new look at physiologic respiratory response to $H_2S$ poisoning. J. Haz. Mat. 13:369-374.

Amoore JE. 1985. The perception of hydrogen sulfide odor in relation to setting an ambient standard. Olfacto-Labs, Berkeley, CA: prepared for the California Air Resources Board.

Amoore JE, Hautala E. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 chemicals in air and water dilution. J. Appl. Toxicol. 3(6):272-290.

Arnold IM, Dufresne RM, Alleyne BC, Stuart PJ. 1985. Health implications of occupational exposures to hydrogen sulfide. J. Occup. Med. 27:373-376.

Beauchamp RO Jr, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA. 1984. A critical review of the literature on hydrogen sulfide toxicity. Crit. Rev. Toxicol. 13(1):25-97.

Beck JF, Cormier F, Donini JC. 1979. The combined toxicity of ethanol and hydrogen sulfide. Toxicol. Lett. 311-313.

Bhambhani Y, Burnham R, Snydmiller G, MacLean I, Martin T. 1994. Comparative physiological responses of exercising men and women to 5 ppm hydrogen sulfide exposure. Am. Ind. Hyg. Assoc. J. 55(11):1030-1035.

Bhambhani Y, Singh M. 1985. Effects of hydrogen sulphide on selected metabolic and cardio-respiratory variables during rest and exercise. Report submitted to Alberta Worker's Health and Safety and Compensation. June, 1985.

Bhambhani Y, Singh M. 1991. Physiological effects of hydrogen sulfide inhalation during exercise in healthy men. J. Appl. Physiol. 71:1872-1877.

Bhambhani Y, Burnham R, Snydmiller G, MacLean I, Lovlin R. 1996. Effects of 10-ppm hydrogen sulfide inhalation on pulmonary function in healthy men and women. J. Occup. Environ. Med. 38(10):1012-1017.

Brenneman KA, James RA, Gross EA, Dorman DC. 2000. Olfactory neuron loss in adult male CD rats following subchronic inhalation exposure to hydrogen sulfide. Toxicol. Pathol. 28(2):326-333.

Cain WS, Stevens JC, Nickou CM, Giles A, Johnston I, Garcia-Medina MR. 1995. Life-span development of odor identification, learning, and olfactory sensitivity. Perception 24(12):1457-1472.

CARB. 1984. California Air Resources Board. Report of the committee regarding the review of the AAQS for hydrogen sulfide. Memorandum from CARB to G. Duffy, August 23.

CARB. 1999. California Air Resources Board. Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29, 1999.

California State Department of Public Health. Recommended Ambient Air Quality Standards. (Statewide standards applicable to all California Air Basins). 1969;HS-3.

CIIT. 1983a. Chemical Industry Institute of Toxicology. 90-Day vapor inhalation toxicity study of hydrogen sulfide in Fischer-344 rats. U.S. EPA, Office of Toxic Substances Public Files. Fiche number 0000255-0. Document number FYI-OTS-0883-0255.

CIIT. 1983b. Chemical Industry Institute of Toxicology. 90-Day vapor inhalation toxicity study of hydrogen sulfide in Sprague-Dawley rats. U.S. EPA, Office of Toxic Substances Public Files. Fiche number 0000255-0. Document number FYI-OTS-0883-0255.

CIIT. 1983c. Chemical Industry Institute of Toxicology. 90-Day vapor inhalation toxicity study of hydrogen sulfide in B6C3F1 mice. U.S. EPA, Office of Toxic Substances Public Files. Fiche number 0000255-0. Document number FYI-OTS-0883-0255.

Dalton P, Wysocki CJ, Brody MJ, Lawley HJ. 1997. The influence of cognitive bias on the perceived odor, irritation and health symptoms from chemical exposure. Arch. Occup. Environ. Health 69(6):407-417.

Dorman DC, Brenneman KA, Struve MF, Miller KL, James RA, Marshall MW, Foster PM. 2000. Fertility and developmental neurotoxicity effects of inhaled hydrogen sulfide in Sprague-Dawley rats. Neurotoxicol. Teratol. 22(1):71-84.

Elovaara E, Tossavainen A, Savolainen H. 1978. Effects of subclinical hydrogen sulfide intoxication on mouse brain protein metabolism. Exp. Neurol. 62:93-98.

Gaitonde UB, Sellar RJ, O'Hare AE. 1987. Long term exposure to hydrogen sulphide producing subacute encephalopathy in a child. Br. Med. J. (Clin. Res. Ed.) 7;294(6572):614.

Haggard HAW. 1925. The toxicology of hydrogen sulphide. J. Ind. Hyg. 7:113-121.

Haggard HW, Henderson Y. 1922. The influence of hydrogen sulfide on respiration. Am. J. Physiol. 61:289-297.

Hannah RS, Roth SH. 1991. Chronic exposure to low concentrations of hydrogen sulfide produces abnormal growth in developing cerebral Purkinje cells. Neurosci. Lett. 122(2):225-228.

Hayden LJ, Goeden H, Roth SH. 1990. Growth and development in the rat during subchronic exposure to low levels of hydrogen sulfide. Toxicol. Ind. Health 6(3-4):389-401.

HAZARDTEXT™. Hall AH, Rumack BH, editors. Denver (CO): Micromedex, Inc.; 1994. (Edition expires 4/30/94).

HSDB. 1999. Hazardous Substances Data Bank. U.S. National Library of Medicine, Bethesda, MD 20894. (http://sis.nlm.nih.gov/sis1)

Hellman TM, Small FH. 1974. Characterization of the odor properties of 101 petrochemicals using sensory methods. J. Air Pollut. Control Assoc. 24:979-982.

Hosoki R, Matsuki N, Kimura. 1997. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem. Biophys. Res. Commun. 237(3):527-531

Hsu P, Li HW, Lin Y. 1987. Acute hydrogen sulfide poisoning treated with hyperbaric oxygen. J. Hyperbaric Med. 2(4):215-221.

Hulbert WC, Prior MG, Pieroni p, Florence Z. 1989. Hyperresponsiveness in rats after 5 weeks exposure to hydrogen sulfide. Clin. Invest. Med. 12(4): B89.

Jaakkola JJ, Vilkka V, Marttila O, Jappinen P, Haahtela T. 1990. The South Karelia Air Pollution Study. The effects of malodorous sulfur compounds from pulp mills on respiratory and other symptoms. Am. Rev. Respir. Dis. 142(6 Pt 1):1344-50.

Jappinen P, Vilkka V, Marttila O, Haahtela T. 1990. Exposure to hydrogen sulphide and respiratory function. Br. J. Ind. Med. 47(12):824-828.

Khan AA, Schuler MM, Prior MG, Yong S, Coppock RW, Florence LZ, Lillie LE. 1990. Effects of hydrogen sulfide exposure on lung mitochondrial respiratory chain enzymes in rats. Toxicol. Appl. Pharmacol. 103: 482-490.

Kilburn KH, Warshaw RH. 1995. Hydrogen sulfide and reduced-sulfur gases adversely affect neurophysiological functions. Toxicol. Ind. Health 11:185-197.

Koelega HS. 1994. Prepubescent children may have specific deficits in olfactory sensitivity. Percept. Mot. Skills 78(1):191-199.

Kosmider S, Rogala E, Pacholek A. 1967. Electrocardiographic and histochemical studies of the heart muscle in acute experimental hydrogen sulfide poisoning. Arch. Immunol. Ther. Exp. 15:731-740.

Landrigan PJ, Miller B. 1983. The Arjenyattah epidemic. Home interview data and toxicological aspects. Lancet 2(8365-66):1474-1476.

Lindvall T. 1970. On sensory evaluation of odorous air pollutant intensities. Nord. Hyg. Tidskr. Suppl 2:1-181.

Lindvall T. 1974. Monitoring odorous air pollution in the field with human observers. Ann. N. Y. Acad. Sci. 237:247-260.

Lindvall T. 1977. Perception of composite odorous air pollutants. In: Olfaction and Taste VI (J. LeMagnen, P. MacLeod, eds.). London: Information Retrieval. pp. 449-458.

Lopez A, Prior M, Yong S, Albassam M, Lillie L. 1987. Biochemical and cytological alterations in the respiratory tract of rats exposed for 4 hours to hydrogen sulfide. Fundam. Appl. Toxicol. 9:753-762.

Lopez A, Prior M, Lillie L, Gulayets C, Atwal O. 1988. Histologic and ultrastructural alterations in lungs of rats exposed to sublethal to lethal concentrations of hydrogen sulfide. Vet. Pathol. 25:376-384.

Marttila O, Jaakkola JJ, Vilkka V, Jappinen P, Haahtela T. 1994. The South Karelia Air Pollution Study: the effects of malodorous sulfur compounds from pulp mills on respiratory and other symptoms in children. Environ. Res. 66(2):152-159.

Modan B, Swartz TA, Tirosh M, Costin C, Weissenberg E, Donagi, A, Acker C, Revach M, Vettorazzi G. 1983. The Arjenyattah epidemic. A mass phenomenon: spread and triggering factors. Lancet 2(8365-66):1472-1474.

NCASI. 1971. Evaluation of the use of humans in measuring the effectiveness of odor control technology at the source. Atmospheric Quality Improvement Technical Bulletin No. 56. New York: National Council of Paper Industry for Air and Steam Improvement.

NIOSH. 1977. National Institute for Occupational Safety and Health. Criteria for a recommended standard...Occupational exposure to hydrogen sulfide, DHEW (NIOSH) #77-158. Cincinnati (OH): National Institute for Occupational Safety and Health; 1977.

NIOSH. 1995. National Institute for Occupational Safety and Health. Chemical listing and documentation of revised IDLH values (as of March 1, 1995). Available at http://www.cdc.gov/niosh/intridl4.html.

National Research Council. Hydrogen sulfide. Baltimore: University Park Press: 1979.

OEHHA. 1999. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part I. The Determination of Acute References Exposure Levels for Airborne Toxicants. Available on-line at http://www.oehha.ca.gov

OEHHA. 2000. Office of Environmental Health Hazard Assessment. Air Toxics Hot Spots Program Risk Assessment Guidelines. Part III. Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Available on-line at http://www.oehha.ca.gov

Prior MG, Sharma AK, Yong S, Lopez A. 1988. Concentration-time interactions in hydrogen sulphide toxicity. Can. J. Vet. Res. 52:375-379.

RTECS®. 1994. Registry of Toxic Effects of Chemical Substances. National Institute of Occupational Safety and Health, Cincinnati (OH) (CD-ROM version). Denver (CO): Micromedex, Inc.; 1994. (Edition expires 4/30/94).

Reiffenstein RJ, Hulbert WC, Roth SH. 1992. Toxicology of hydrogen sulfide. Annu. Rev. Pharmacol. Toxicol. 32:109-134.

Reynolds R L, Kamper RL. 1984. Review of the State of California Ambient Air Quality Standard for Hydrogen Sulfide ($H_2S$). Lakeport (CA): Lake County Air Quality Management District; 1984.

Saillenfait A, Bonnet P, DeCeaurriz J. 1989. Effects of inhalation exposure to carbon disulfide and its combination with hydrogen sulfide on embryonal and fetal development in rats. Toxicol. Lett. 48:57-66.

Schmidt HJ, Beauchamp GK. 1988. Adult-like odor preferences and aversions in three-year-old children. Child. Dev. 59(4):1136-1143.

Simson RE, Simpson GR. 1971. Fatal hydrogen sulphide poisoning associated with industrial waste exposure. Med. J. Austral. 2:331-334.

Spiers M, Finnegan OC. 1986. Near death due to inhalation of slurry tank gases. Ulster Med. Soc. 55(2):181-183.

Tansy MF, Kendall FM, Fantasia J, Landlin WE, Oberly R, Sherman W. 1981. Acute and subchronic toxicity of rats exposed to vapors of methyl mercaptan and other reduced-sulfur compounds. J. Toxicol. Environ. Health 8(1-2):71-88.

U.S.EPA. 1999. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for hydrogen sulfide. Available on-line at http://www.epa.gov/ngispgm3/iris/subst/index.html

Venstrom P, Amoore JE. 1968. Olfactory threshold in relation to age, sex or smoking. J. Food Sci. 33:264-265.

Winkler K. 1975. Zur Diskussion Gestellt Imissionsgrenzwerte Zur Vehrinderung von. Geruchsbelastigungan. Wasser Luft Betrieb. 19:411.

Winneke G, Kastka J. 1977. Odor pollution and odor annoyance reactions in industrial areas of the Rhine-Ruhr region. In: Olfaction and Taste VI. J Le Magnen, P MacLeod, editors. pp. 471-479. London: Information Retrieved.

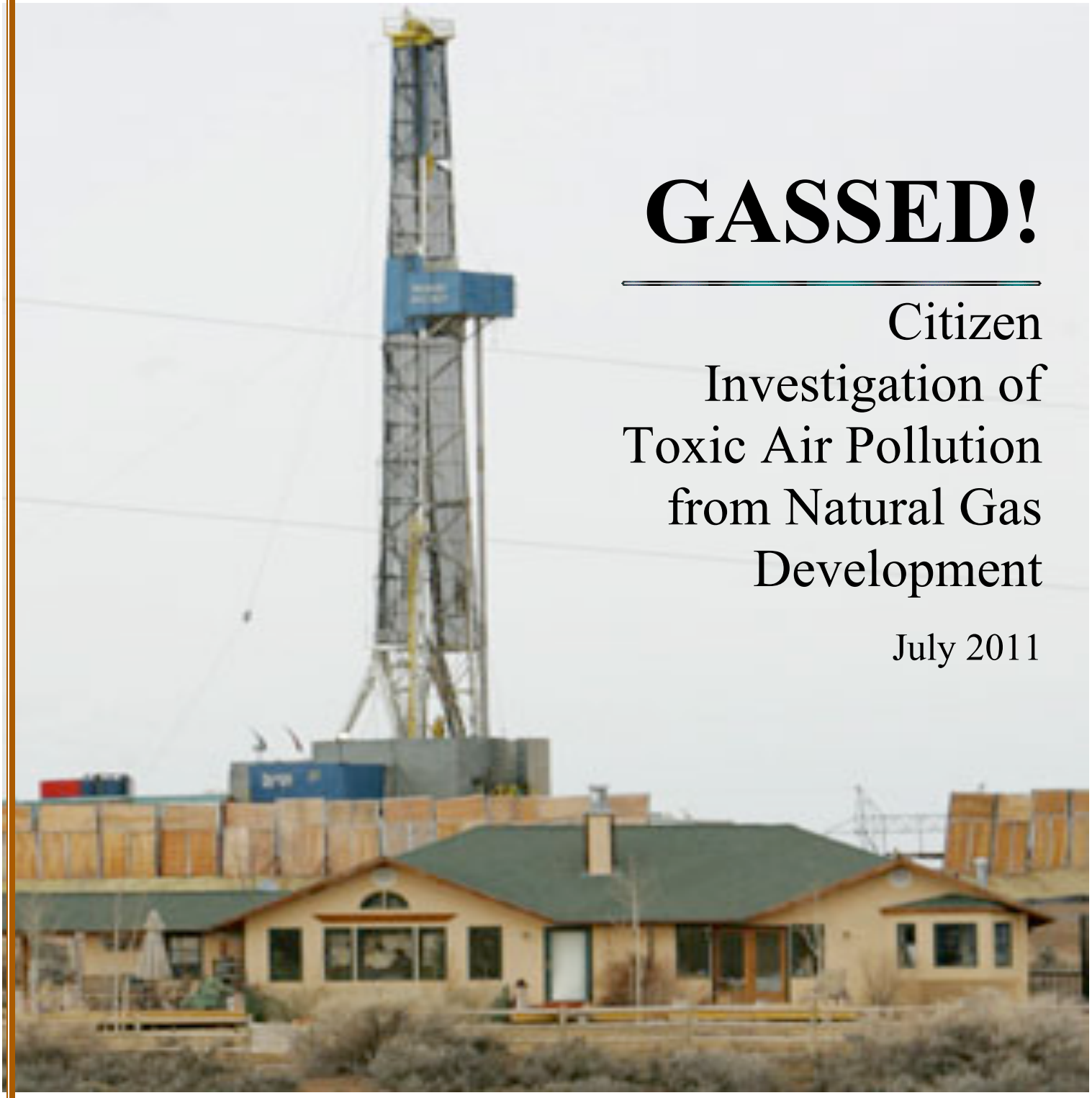
(WHO) World Health Organization. Hydrogen sulfide. Environmental Health Criteria No. 19. Geneva: WHO; 1981.

Xu X, Cho SI, Sammel M, You L, Cui S, Huang Y, Ma G, Padungtod C, Pothier L, Niu T, Christiani D, Smith T, Ryan L, Wang L. 1998. Association of petrochemical exposure with spontaneous abortion. Occup. Environ. Med. 55(1):31-36.

# GASSED!

## Citizen Investigation of Toxic Air Pollution from Natural Gas Development

July 2011



10th ANNIVERSARY

Global Community Monitor

Breathing New Life into Communities

**gcmonitor.org**

## Background on Global Community Monitor

Global Community Monitor, founded in 2001, trains and supports communities internationally in the use of environmental monitoring tools to understand and address industrial toxic pollution threats to their health and the environment.

GCM, best known as the innovator of the "Bucket Brigade", incubates community-based groups to develop the skills, expertise, and experience to win demands around environmental health and justice. Since GCM's approach is extremely replicable and effective, we have been invited to work with more than 40 communities in 27 countries. GCM collaborates with an established network of environmental health experts in the US and internationally to leverage resources for the communities.

Addition Information including News and Media available at:
http://gcmonitor.org/section.php?id=179
http://gcmonitor.org/section.php?id=29
http://gcmonitor.org/section.php?id=224


## *Special Thanks To*

***The Natural Resources Defense Council (NRDC)***
***Funds from the William and Flora Hewlett Foundation helped make the monitoring possible***
***Western Colorado Congress (WCC)***
***San Juan Citizen Alliance (SJCA)***
***All The Community Monitors of Aztec, Durango, Battlement and Silt Mesa***
***Kresge Foundation***

Report Contributors:

Denny Larson, Ruth Breech,
Jessica Hendricks, Mark Chernaik,
Amy Mall, Frank Smith, Josh Joswick,
Mike Eisenfeld

Global Community Monitor
PO Box 1784
El Cerrito, CA 94530
Gcmonitor.org
info@gcmonitor.org

# GASSED!

Citizen Investigation of Toxic Air Pollution from Natural Gas Development

**Table of Contents**

**Executive Summary** *pg. 2*

**Oil and Natural Gas Development and Air Pollution** *pg. 5*
- a) Life Cycle of Natural Gas Development
- b) Air Pollution and Human Health Impacts of Natural Gas Development *pg. 8*

**Natural Gas Development in Colorado and New Mexico** *pg. 10*
- a) Growth in Project Area
- b) Target Communities *pg. 11*

**Citizen Air Sampling and Results: Bucket Brigade Project** *pg. 16*
- a) Community-Based Air Monitoring: Crucial Piece to the Puzzle
- b) Bucket Brigade Training and Methods *pg. 16*
- c) Results and Discussion of Results *pg. 19*

**Recommendations** *pg. 25*

**Appendix**

## Executive Summary

Over the past decade, oil and natural gas exploration and production have grown at an unprecedented rate in the United States. Since necessary environmental and health regulations are not in place for this industry, residents living near oil and natural gas sites may be exposed to highly toxic chemicals on a regular basis, with their health at risk.

During 2010-11, Global Community Monitor (GCM), responding to citizen odor and health complaints, launched a community-based pilot environmental monitoring program in northwest New Mexico, southwest Colorado and western Colorado to document and measure air pollution from natural gas facilities. Through the course of this pilot study, residents, armed with their own air monitors, documented a potent mix of chemicals in nine air samples from different locations. The sites in this program are all natural gas production and processing sites, although production of oil presents similar risks. Air sampling for this project targeted many aspects of natural gas development.

Through the course of this study, several serious issues emerged:

**Citizen samples exposed alarming levels of toxins in the air**.

A total of *22 toxic chemicals* were detected in the nine air samples, including *four known carcinogens,* toxins known to damage the nervous system, and respiratory irritants. The levels detected were in many cases significantly higher than what is considered safe by state and federal agencies. The levels of chemicals, including benzene and acrylonitrile, ranged from three to 3,000 times higher than levels established to estimate increased risk of serious health effects and cancer based on long-term exposure.

These air samples confirm the observations, experiences and first-hand complaints of residents. Odors and health effects that have been reported for years were consistent with exposure to the chemicals found in the samples. These results underscore the need of regulatory agencies to take such complaints seriously, given the close proximity between the industry and its residential neighbors.

At least two cancer-causing chemicals, acrylonitrile and methylene chloride, were detected at high levels near natural gas operations. Neither chemical is associated with natural gas or oil deposits, but both seem to be associated with the use of hydraulic fracturing (fracking) products. Resins acrylonitrile, 1, 3 butadiene and styrene (ABS) are suspected to be present in fracking additives.

**Air emissions from natural gas production are largely unregulated and unmonitored**, despite being a significant source of air pollution. State and Federal air monitoring devices are located several miles from production sites, and test for criteria air pollutants rather than specific volatile organic compounds associated with natural gas exploration and production.

Oil and gas exploration and production operations are exempt from two key provisions of the Clean Air Act's National Emissions Standards for Hazardous Air Pollutants, designed to protect

public health. Because of these exemptions, the industry avoids complying with standards that are applied to other industries.

Based on the data gathered in this pilot study, highly toxic chemicals are permeating the air near homes, farms, schools, playgrounds, and town centers. Due to the lack of regulation and standards, key information about chemicals being used in the production process, including hydraulic fracturing is widely unavailable. Combined with the lack of appropriate air monitoring near production sites, **citizen right-to-know is virtually non-existent.**

Without registration of the chemicals by industry, neighbors of gas wells have no way of knowing what chemicals are stored on site, used during the industrial processes, vented to the air, water or land, or disposed nearby.

**Recommendations**

1. Given the proximity of residential and public property, any new sites –whether drilling, fracking, refining, or disposal – should be located at least one-quarter mile from homes, farms, schools, playgrounds, and businesses. This space would provide a **buffer zone** for industry to continue its operations while reducing community exposure to chemical contaminants.

2. The U.S. Environmental Protection Agency (EPA) should update air quality standards for oil and gas development, including the New Source Performance Standards and National Emission Standards for Hazardous Air Pollutants, based on the principles of comprehensiveness, effectiveness, full health protection, forward looking, and enforceability.

3. Until strong new rules are in place, the oil and natural gas industry can and should **voluntarily invest in equipment that reduces pollution escaping to the air**. Such equipment is readily available and financially profitable for companies. These investments would increase efficiency and production and reduce cancer-causing chemicals from being emitted into the air in communities near production facilities – saving lives and protecting the health of neighboring families.

4. Current natural gas production and processing sites should **have air monitors near all operations and equipment. All data should be made available to the public**.

5. **EPA and state agencies must enforce the current laws** on the books vigorously and impose the maximum penalties available to create a culture that prioritizes public health. Regulators should be accessible and fully funded to ensure their ability to protect public health and the environment.

As the natural gas industry continues to grow, so will the number of families neighboring and affected by the emissions. Industry and government leaders have a unique opportunity to address public health and environmental issues by implementing all of these recommendations. For

coexistence between communities and industry to be possible, chemical exposure has to be immediately addressed.

## Oil and Natural Gas Development and Air Pollution

There are a variety of chemicals used and released during the drilling, fracking, and production phases of oil and gas development. In addition, different types of wastes are produced throughout the development process. Air pollution is generated at all stages of oil and gas development including wellpad construction and drilling, workovers, fracking and completion, gas compression, evaporation of chemicals from produced water and frack flowback, dehydration, separation, waste treatment and disposal, transmission and processing.

The following is a brief glossary of the life cycle of natural gas development:

### Construction activity

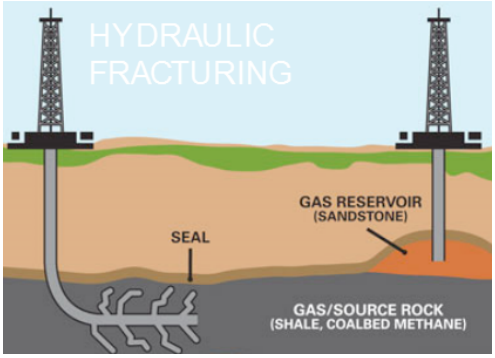
Even prior to producing natural gas, air pollution is generated by heavy construction activity including trucks and other equipment that emit air pollutants at well pads, pipelines, roads and compressor stations.

### Drilling

During the drilling of a well, air pollution is generated by diesel engines powering the drill rig, as well as by any natural gas emissions being vented from the hole in the ground. These emissions could include various toxic gases, including volatile organic compounds.

### Hydraulic fracturing (fracking) and completion[1]

*Image from Stark Political Report*




While oil and gas have always been extractable from the natural fissures in certain rock formations, some of these deposits are too diffuse to be economically feasible to exploit using traditional drilling methods. Increasing demand, however, has spurred the evolving development of fracturing technology. Pioneered in west Texas, fracking is being used to increase the productivity of drill sites in shale, coalbed methane, and tight sands formations that previously were too expensive to drill.

Fracking is dependent on fracturing fluid, typically comprised of water-based concoctions riddled with assortments of chemicals and proppants like sand. The chemical makeup of the fluid varies from company to company and site to site. The process of fracking involves perforating oil and gas wells and then pumping chemical fluid into the earth. By pumping fracturing fluid deep into the rock formation fissures under the earth at very high pressure, the cracks are expanded and then propped open with the proppant. These expanded cracks allow a single well to tap into multiple diffuse deposits.

---

[1] "Hydraulic Fracturing Research Study," US EPA. Office of Research and Development. 24 May 2011. http://www.epa.gov/safewater/uic/pdfs/hfresearchstudyfs.pdf

**Though fracking enables cost effective production of natural gas for the gas companies, it also comes with risks to public health and the environment.** One of the least documented risks has been from air pollution caused by fracking compounds during their use, storage, or waste disposal.

**Pits**
Waste from drilling, fracking, or production may be dumped into open air pits to allow some of the toxic material to evaporate into the air. This can result in significant air pollution.

**Land application (including land farming)**
Waste from drilling, fracking, or production may be spread on the ground or otherwise applied to the land. This can result in significant air pollution.

**Compressor station**
Gas from wells is collected at central locations and compressed into smaller volumes at stations. Another type of compressor is located on the well site. Both types of compressors can leak and release a variety of toxic gases.

**Condensate tanks**
Some well sites produce semi-liquid gases along with natural gas that are stored in tanks, which can leak various toxic gases into the air.

**Dehydrators**
These systems are needed to remove water from natural gas and can release toxic gases in the process.

**Flaring**
Unwanted gases in the production process may be burned off in the open air through flares, which can produce other toxic gases as a result.

**Fugitive emissions**
Leaks in equipment such as pumps, valves, compressors, pipes and tanks can result in significant air pollution releases because of the number of components in gas processing.

**Venting**
During various stages of gas exploration, production and maintenance, gases are vented directly into the air rather than contained or flared. Venting can release large volumes of toxic gases.

**Gas processing plant**
The last stage of gas production involves the refining of the raw gas into the final product. This occurs at large gas processing plants, which have many sources of air emissions.

**Additional waste disposal sites**[2]

Wastes from various stages of gas production and processing may be sent to treatment sources including landfills, injection sites and wastewater treatment sites, which can also release air pollution.

[2] "Public Health and Toxics." EARTHWORKS. 20 March 2011, http://www.earthworksaction.org/Health%20and%20Toxics.cfm

## Air Pollution and Human Health Impacts of Natural Gas Development

Air pollution can affect our health in many ways, with both *short-term* and *long-term* effects. Different groups of individuals are affected by air pollution in different ways. Some individuals are much more sensitive to pollutants than others. Sensitive populations, including young children and elderly people, often suffer more from the effects of air pollution. People with health problems such as asthma, heart and lung disease may also suffer more when the air is polluted. The extent to which an individual is harmed by air pollution usually depends on the **total exposure** to the damaging chemicals, i.e., the *duration of exposure* and the *concentration of the chemicals.* Total exposure must be taken into account when assessing air pollution risks.

Examples of **short-term effects** include irritation to the eyes, nose, and throat, and upper respiratory infections such as bronchitis and pneumonia. Other symptoms can include headaches, nausea, and allergic reactions. Short-term air pollution can aggravate the medical conditions of individuals with asthma and emphysema.

**Long-term health effects** can include chronic respiratory disease, lung cancer, heart disease, and even damage to the brain, nerves, liver, or kidneys. Continual exposure to air pollution affects the lungs of growing children and may aggravate or complicate medical conditions in the elderly.[3]

Chemicals such as benzene, toluene, ethylbenzene and xylene (BTEX) are known to be present around natural gas development sites, both from the gas deposits as well as chemical additives. Our independent testing found significantly high amounts of these toxic gases downwind of various sites. Health effects from BTEX include dizziness and confusion, eye, nose and throat irritation, birth defects, kidney, liver, and neurological damage, and cancer. For example, benzene is known to cause leukemia.[4]

Hydrogen sulfide was also found in the Bucket tests, warning signs for the gas are often visible near well pads. Long-term exposure to hydrogen sulfide is associated with an elevated incidence of respiratory infections, irritation of the eye, nose and throat, coughing, breathlessness, nausea, headache, and mental health impacts, including depression.[5] It is recommended, that workers handling hydrogen sulfide be equipped with hydrogen sulfide monitors, respirators, and rescue packs for protection in the event of elevated exposure; the public has no such protection.[6]

Additional toxic substances were detected at high levels in the air samples, including toxic gases not previously associated with natural gas development, suggesting that substances possibly associated with fracking additives may have been released into the air.

---

[3] "How Can Air Pollution Hurt My Health." Health Effects of Air Pollution, Lawrence Berkeley National Laboratory, March 2011, http://www.lbl.gov/Education/ELSI/Frames/pollution-health-effects-f.html
[4] NRDC, Drilling Down, October, 2007, table on page vi
[5] Chernaik, Mark. Data Interpretation Synthesis Letter. Science for Citizens. 16 Feb. 2011
[6] Air Products, Material Data Sheet, http://avogadro.chem.iastate.edu/MSDS/hydrogen_sulfide.pdf

Overall, air samples gathered for this project showed that neighbors of the natural gas operations in the target communities are breathing multiple chemicals that can cause an increased risk of cancer and other serious health effects. There are no health-based standards for exposure to multiple chemicals, although the negative health impacts are considered to be increased.

## Natural Gas Development in Colorado and New Mexico

### Growth in Project Areas

GCM worked with two communities in the San Juan Basin—one in southwest Colorado and a second in northwest New Mexico. In addition, GCM worked with a third community in Garfield County in western Colorado.

- ***Colorado's natural gas production has risen 450% since 1990 with over 27,000 active wells statewide.*** [7]

- ***Currently there are approximately 3,400 wells in La Plata County, CO.*** [8]

*Image from BP.com*

- ***There are approximately 21,000 wells in San Juan County, NM***

  - ***The approximate total of wells in the entire San Juan Basin is 35,000 wells*** [9]

*Image from Realtor.com*

- ***In western Colorado, Garfield County has an estimated 8,249 active wells with 2,037 new permits approved in 2010*** [10]

---

[7] "Background." Western Colorado Congress, 20 March 2011, http://www.wccongress.org/gvca.htm#background
[8] "Natural Resources- Oil and Gas." La Plata County Planning Department, 25 March 2011, http://www.co.laplata.co.us/departments_and_elected_officials/planning/natural_resources_oil_gas
[9] United States Department of Interior, Bureau of Land Management, **Farmington Resource Management Plan** (December 2003) Final **RMP**/Record of Decision
[10] May 2011 Colorado Oil & Gas Conservation Commission Staff Report

## Target Communities

GCM worked with communities in northwest New Mexico and southwest Colorado in partnership with the San Juan Citizens Alliance. The project also included communities in western Colorado in partnership with the Western Colorado Congress. The communities were trained in air monitoring and bucket sampling around natural gas development sites.

Northwest New Mexico: Aztec and Farmington Area

Of the three communities involved in this pilot project, northwest New Mexico has the longest history of complaints about natural gas drilling. Natural gas has been drilled for, and produced, in northwest New Mexico for over 60 years with natural gas facilities interspersed among residential areas. Community residents in northwest New Mexico have noticed strong odors since the late 1980s, reported as smelling like rotten eggs, petroleum and sewage, around the ever-expanding oil and gas industry. Residents have experienced nose, throat and eye irritation that occasionally would last for hours after smelling the odors. When the odors increased in frequency, so did the associated acute health effects.

Energy companies in the area, including BP, Energen, XTO, Devon, Conoco Phillips, Enterprise, Williams and Questar, are associated with drilling for and transporting natural gas, where operations at sites can include fracking by numerous companies. San Juan County in Northwest New Mexico consists of more than 100,000 residents potentially affected by natural gas production, either by living near a gas well or near the plants that process the natural gas.

There are many gas wells near schools, churches, private residences and community centers. Natural gas odor incidents are frequent, along with adverse health effects in the community. For example, in December 2009, one of the members of the San Juan Citizens Alliance and long-term area resident Shirley McNall went out to get her mail. She was immediately struck with an extremely potent rotten egg odor and overcome with dizziness and nausea. According to McNall, she fell to the ground and was forced to crawl back into the house. While the symptoms began to slowly subside, she reported numbness in her lips that lasted for three days after the incident.

During less severe odor incidents, residents commonly reported headaches, nausea and dizziness in addition to nose, throat and eye irritation.

***Shirley McNall- Aztec, NM***

The health effects and reported odors could be associated with chemical exposure. McNall and other residents have documented odors most frequently during the late evening through the early morning hours. This could be related to the industrial process and/or weather patterns that concentrate or bring the toxic fumes near homes.

Community members call the New Mexico Oil and Gas Conservation Division frequently, often multiple times a week, to report these odors. However, no satisfactory permanent solutions have been reached. On occasion, a representative of the New Mexico Oil and Gas Conservation Division will conduct an on-site investigation. During one of these investigations, the representative informed the residents that the most likely cause of the odors is "treated" hydrogen sulfide. This is a major concern because hydrogen sulfide is highly toxic and, while its presence requires formal signage by law, no signage was present at the well under investigation.

Homeowners are not generally informed of the toxic risk when their property is in proximity to natural gas facilities. Split estate situations where mineral ownership is separate from private surface ownership creates confusion and uncertainty surrounding where wells can be drilled in relation to homes. Numerous contractors and subcontractors may be involved with natural gas facilities, further complicating responsibilities and actions. The New Mexico Environmental Department and the U.S. Environmental Protection Agency's (EPA) efforts to monitor and evaluate air impacts from natural gas resources in northwest New Mexico have been limited.

Southwest Colorado: Durango

This troubling trend is not unique to the northwest corner of New Mexico. The expanding oil and gas industry has spread into communities in Colorado.

La Plata County, located in southwest Colorado where the southern Rockies meet the high desert country of northern New Mexico, is home to three municipalities, four river drainages, and a sovereign Indian nation. It is known for its outdoor activities including hiking, rock climbing, backpacking and white water rafting along the Animas River, and for the incredibly lucrative coalbed methane field that underlies it.

Coalbed methane development has been going on here since in the mid-1980s. The environmental degradation associated with development has been documented to include coal seam fires and hydrogen sulfide and methane seeps at the Fruitland formation outcrop. The full impacts of development on air quality and public health, however, remain largely unexamined.

Due to split estate status, energy companies can lease the mineral rights underneath the property of a homeowner. Insufficient setbacks and surface owner protections allow the oil and gas industry to place facilities directly next to homes and schools. Near Sunnyside Elementary School, air monitoring on January 7, 2011 showed elevated levels of four known carcinogens. Two of the carcinogens were recorded at levels that are considered to be an unacceptable long-term exposure risk.

*Josh Joswick- Durango, CO*

LaPlata County has an estimated 3,400 wells. Many county residents therefore live in or adjacent to the 'gas patch,' often times in close proximity to gas wells, compressor stations, dehydrators, and processing plants. This incompatible mix of industrial activity in rural residential areas has had an impact on

people's lives. Gas patch residents in La Plata County report odors similar to their neighbors in New Mexico. These odors, smelling like burning oil, car exhaust, and burning rubber, are most frequently noticed around well sites.

Aaron Mallet, a La Plata County resident active with the Bucket Brigade, stated on September 28, 2010: "On a regular basis there is an acrid smell in the air that emanates from that well pad."[11] Residents have also documented headaches, sore throats and burning nasal passages during these odor incidents.

Western Colorado: Battlement and Silt Mesas

Lastly, GCM worked with the communities of Battlement and Silt Mesas in Garfield County, Colorado. Battlement and Silt Mesas are two rural communities experiencing impacts from nearby development of natural gas.

Battlement Mesa is an unincorporated retirement community of 5,500 residents in western Garfield County. Originally constructed by Exxon in the 1970s for workers in the oil shale industry,[12] it was later marketed as a destination for retirees seeking a peaceful place to spend their golden years. Exxon eventually sold the surface properties but retained the mineral rights to extract the fossil fuels beneath Battlement Mesa at any time in the future.

Community members watched as natural gas wells incrementally came closer to Battlement Mesa, and the residents began to wonder if drilling would be allowed within their retirement neighborhood.

*Dave Devanney- Battlement Mesa, CO*

In 2009, Battlement Mesa learned of a proposal to drill 200 natural gas wells within its borders, including sites near homes, along the Colorado River, on the golf course, and near a school. Battlement Mesa residents called for thorough scientific research of the potential public health impacts of natural gas development before any permitting decision. After hundreds of residents signed a petition, a groundbreaking Health Impact Assessment was commissioned for drilling within Battlement Mesa and county officials delayed any new drilling inside of the retirement community until this process was completed. Drilling, however, began just outside the border of the community and community members began complaining of noxious fumes being emitted.

Battlement Mesa residents documented strong petroleum-like odors in the middle of the night and early mornings. Residents believed that these strong petroleum, diesel and chemical smells were caused by nearby fracking operations. Nearby residents began experiencing health effects such as throat and nose irritation, headaches, itching skin, burning eyes, and dizziness. Residents

[11] Mallet, Aaron. Pollution Log 28 Sept. 2011
[12] Oil Shale is a different formation than the source of shale gas.

called the Colorado Oil and Gas Conservation Commission to formally report the odor events; they started documenting odor occurrences, and they contacted local authorities.

The Colorado Oil and Gas Conservation Commission cited the operator for failing to capture nuisance odors derived from its operations. The company was encouraged to use additional vapor recovery techniques during flowback operations to reduce odors (but never received a monetary penalty). Residents noticed a marked diminishment of the odors, but around the same time, in November of 2010, a local news channel highlighted nearby Silt Mesa residents' problems with natural gas development. Silt Mesa residents reported odors they thought were caused by natural gas activity, and Dave Devanney of the Battlement Concerned Citizens contacted them.

Silt Mesa is a network of irrigation canals and small ranches, sitting along the Colorado River between Rifle and Silt, Colorado. Drilling for natural gas is taking place near homes and water supplies, presenting many of the same challenges as on Battlement Mesa.

One Silt Mesa family with two young sons had three natural gas drill rigs surrounding its property, each with ongoing flaring. The nearest flare stack was less than one-half a mile from their home. Family members reported pungent odors of rotten eggs followed by severe headaches, nosebleeds and rashes. The nosebleeds were persistent and heavy, much different than the average nose bleed. The mother described it as "almost like hemorrhaging." The youngest son developed a full body rash, which prompted a doctor visit. Upon examination, the doctor immediately told the Silt Mesa family to evacuate their home.

Although the family was forced to vacate their home because of nearby industrial activity, the state did not issue any violations. According to Colorado rules, Silt Mesa is not a High Density area, therefore, drilling for natural gas in the area does not warrant additional safety precautions.[13]

Today, the Silt Mesa family has left their home and put it up for sale. An air sample taken on January 15, 2011, on their property, contained levels of hydrogen sulfide more than 185 times above the long term level set by the U.S. EPA (2 $\mu g/m^3$) to estimate increased risk of serious health effects.

This Silt Mesa family, as well as the Battlement Mesa residents, call frequently to report odor complaints and other incidents of non-compliance. They call the Colorado Oil and Gas Conservation Commission, the Garfield County Oil and Gas Department, the Colorado Department of Public Health and Environment, and occasionally, the Environmental Protection Agency. The communities have seen worse local air quality since natural gas development markedly increased in Garfield County, although limited air monitoring is conducted by local and state authorities.

Collectively, nine air samples were taken by the Bucket Brigades. The members of San Juan Citizens Alliance and Battlement Concerned Citizens have taken the results to local officials and

[13] Colorado Oil and Gas Conservation Commission. "Series Safety Regulations" 2 June 2011. http://cogcc.state.co.us/

the U.S. EPA, but, to date, the agencies have not taken any action. Most of the residents feel their concerns have fallen on deaf ears.

A press release was issued in Aztec, New Mexico and Durango, Colorado announcing the air sample results. The residents still have not received an adequate response from the regulatory agencies. On Monday, April 4, 2011, Katee McClure sent an e-mail to the New Mexico Environment Department inquiring about who is responsible for enforcing air regulations. Although, the agency did respond in a timely manner, it provided incorrect information regarding standards for hydrogen sulfide pollution while failing to take responsibility or provide information for the responsible agency.[14]

[14] McClure, Katee. 4 April 2011

## Citizen Air Sampling: Bucket Brigade Projects

Community-Based Air Monitoring: A Crucial Piece of the Puzzle

*Building a trail of evidence*

Regulatory and environmental agency personnel are not available at all hours to come out during a pollution incident. In the case of Colorado and New Mexico, a proper citizen complaint system is not established. A proper citizen complaint system would include a telephone hotline followed by rapid response from regulatory agencies and timely air sampling during odor incidents. Community-based monitoring provides an opportunity for residents to respond immediately to the pollution incident with sampling equipment and to contact agency personnel.

GCM trained members of the Western Colorado Congress, the San Juan Citizens Alliance, and other community members to keep a record of pollution incidents. These records include: the location, nature, and duration of the incident; the wind direction, health effects or property damage; and how the incident was addressed – by a call to the regulatory agency or the company suspected or known to be the source of the pollution, or informative calls to other neighbors.

Pollution incident records are referred to as "pollution logs." Pollution logs filled out by community members ensure that a record is maintained beyond regular agency business hours. Community members are also encouraged to take pictures and/or use a video camcorder to catch a visual image of the pollution.

Bucket Brigades provide evidence and hard science to support the anecdotal stories of health impacts that all affected communities know too well: strange odors causing nausea, stinging eyes, burning noses, sore throats, coughs, and other distressing health symptoms. Community-based monitoring engages community members in record maintenance, site identification, operation of monitoring equipment, documentation, and custody and shipping of the sample.

The information gathered by Bucket Brigades, combining science with community experience and reports, helps bridge the gap between communities, regulators and industry. Air sampling and monitoring can provide key evidence exposing chemical exposure, can be a tangible way to show that the air pollution has decreased in a community, and can help build relationships where community members coexist with their industrial neighbors.

**Bucket Brigade Training & Methods**

To begin a project, GCM conducts a research assessment of toxic hazards in a target community and identifies the appropriate environmental monitoring tools that will assist community members in investigating their health concerns and exposures. We review the data on pollution sources and toxins and prioritize the most serious for early action. Due to the lack of publicly available data regarding the air emissions from natural gas drilling and refining sites, we had little research available for reference in this project.

All Bucket Brigade trainings are conducted on site, in the local community. For this project,

GCM was given a local tour by community members in areas near Durango, Colorado; Battlement/Silt Mesas, Colorado; and Aztec, New Mexico in late July, 2010. During the training, GCM provided a day-long classroom training, including background on pollution and environmental health, how to document pollution incidents, hands on training and how to use monitoring equipment. We worked with the local community members to co-design an environmental sampling plan.

The training and plans emphasize standard scientific methods. Community members learn how the monitoring equipment works, the best time to use it, and the appropriate paperwork to fill out before shipping a sample to the lab. The Bucket Brigade's work is strengthened by following stringent Quality Assurance/Quality Control (QA/QC) protocols and the use of EPA approved labs.

*The Bucket Monitoring Equipment*

Due to the nature of the uncertainty of the emissions associated with natural gas drilling, hydraulic fracturing and refining, this project chose to use the Bucket as the monitoring equipment. The Bucket is modeled after the Summa Canister,[15] but has some advantages in its use.

The Bucket is portable, requiring only a tedlar bag and vacuum to take the grab sample. Air is "grabbed" out of the air for two to three minutes and captured in the bag. Once the sample is taken, the tedlar bag is sealed, removed from the bucket and sent to the lab for analysis.

The air sampling Bucket, gcmonitor.org

[15] State of Nevada, Division of Environmental Protection. "Summa Canister Sampling". http://ndep.nv.gov/fallon/summa.pdf

The lab analysis is conducted by Columbia Analytical Services in Simi Valley, California. The lab utilizes EPA method TO-15 and ASTM D 5504-08 method for sample analysis. The TO-15 analysis includes a spectrum of more than 70 volatile organic compounds and the ASTM D 5504-08 method is used to test for 20 sulfur compounds.

Once the community members are trained on the equipment, the buckets are kept at various locations in the community – selected based on the location of odors and health symptoms that have been experienced and reported. When an odor incident occurs, Bucket Brigade members join together to bring a bucket to the site of the odor incident and take a sample of the air at the time of the odor.

## Results & Discussion of Results

## Individual sample results and overall trends:

For this project, communities in New Mexico and Colorado took a total of nine air samples between September 2010 and January 2011. This report documents serious toxic air pollution generated at various points of the life cycle of natural gas development. Targeted sampling sites included well pad, compressor station, gas separation plant, dehydrator and waste disposal site. Serious cancer-causing chemicals were detected at elevated levels, including chemicals associated with the fracking process used increasingly by energy companies.

While bucket samples are short-term grab samples of the air breathed by community members living near natural gas development facilities, letters and pollution logs reveal that the odors are persistent and occur on an ongoing basis. We therefore consider the data to be indicative of long-term exposures, and the expert interpretation used in this report compares the data to pollutant levels linked to long-term health effects.

A total of 22 toxic chemicals were detected in the nine air samples, including four known carcinogens, toxins known to damage the nervous system, and respiratory irritants. The levels of chemicals detected were in many cases significantly higher than is considered safe by state and federal agencies. The levels were between three to 3,000 times higher than levels established by public health agencies to estimate increased risk of serious health effects and cancer based on long-term exposure.[16]

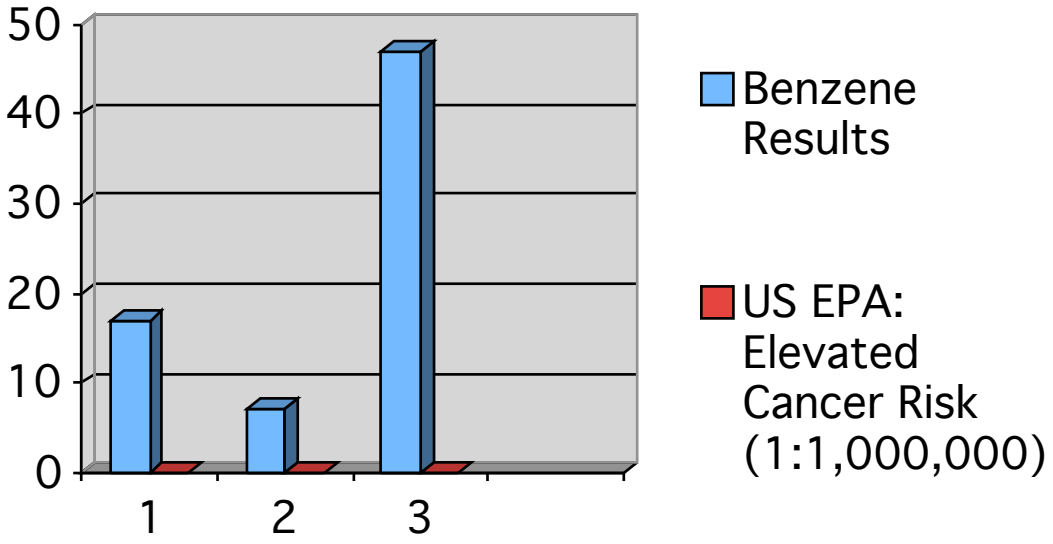
The most significant results:

- ***Benzene***, a known carcinogen, was found at high concentrations in four air samples at levels between 6.3 and 47 $\mu g/m^3$. These levels are 48.5 to 800 times higher than the level set by the US EPA of 0.13 $\mu g/m^3$ to estimate increased cancer risk from long-term exposure.[17]

  Benzene can also cause serious non-cancer health effects which can damage the blood and nervous system Levels of benzene in one of the nine samples, collected on January 7, 2011 near the Sunnyside Elementary School, Durango, Colorado, exceeded the level set by the U.S. EPA for benzene (30 $\mu g/m^3$) to estimate increased risk of non-cancer health effects.

[16] This report defines an elevated cancer risk as 1:1,000,000
[17] http://www.epa.gov/ttn/atw/toxsource/table1.pdf

**Sample 1:** 200 Montana St Bloomfield NM
**Sample 2**: Bondad 33-10 #26 Williams Well, Durango, CO
**Sample 3:** Intersection of US 550 & CR 218 Durango, CO, near Sunnyside Elementary




- ***Acrylonitrile***, a human carcinogen, was found in five samples at levels between 7.9 and 30 μg/m$^3$. These levels are 790 to 3000 times above the U.S. EPA level of 0.01 μg/m$^3$, set to estimate an increased risk of cancer from long term exposure. All of these levels correspond to what EPA would consider an "unacceptable cancer risk" in that long-term exposure is associated with a cancer risk of greater than 100 in a million.[18]

  Acrylonitrile is also a respiratory irritant, causing degeneration and inflammation of nasal epithelium. Levels of acrylonitrile in the five samples exceeded the level set by U.S. EPA for risk of increased non-cancer health effects from long term exposure (2 μg/m$^3$) by 3 to 15 times.[19]

- ***Methylene chloride***, a human carcinogen, was found in five samples at levels between 7.9 and 17 μg/m$^3$. These levels are 3 to 8 times higher than the level set by the U.S. EPA (2.0 μg/m$^3$.) to estimate an increased risk of cancer from long-term exposure.

[18] Communication from Miriam Rotkin-Ellman, Natural Resources Defense Council. 7 June 2011
[19] The USEPA Reference Concentration (RfC) is an estimate of a continuous inhalation exposure concentration to people (including sensitive subgroups) that is likely to be without risk of deleterious effects during a lifetime.

- ***Ethylbenzene***, a human carcinogen, was found in five samples at levels between 5.1 to 22 μg/m$^3$. These levels are 12 to 55 times higher than the level set by the US EPA (0.4μg/m$^3$) to estimate increased cancer risk cancer from long-term exposure.

- ***Xylene***, were found at a level of 100 and 154 μg/m$^3$. These levels exceed the U.S. EPA's level for estimating increased non-cancer health risks of 100 μg/m$^3$.

- ***Hydrogen sulfide*** was found in one sample at 370 μg/m$^3$ which is more than 185 times above the long term level set by the U.S. EPA (2 μg/m$^3$) to estimate increased risk of serious health effects.

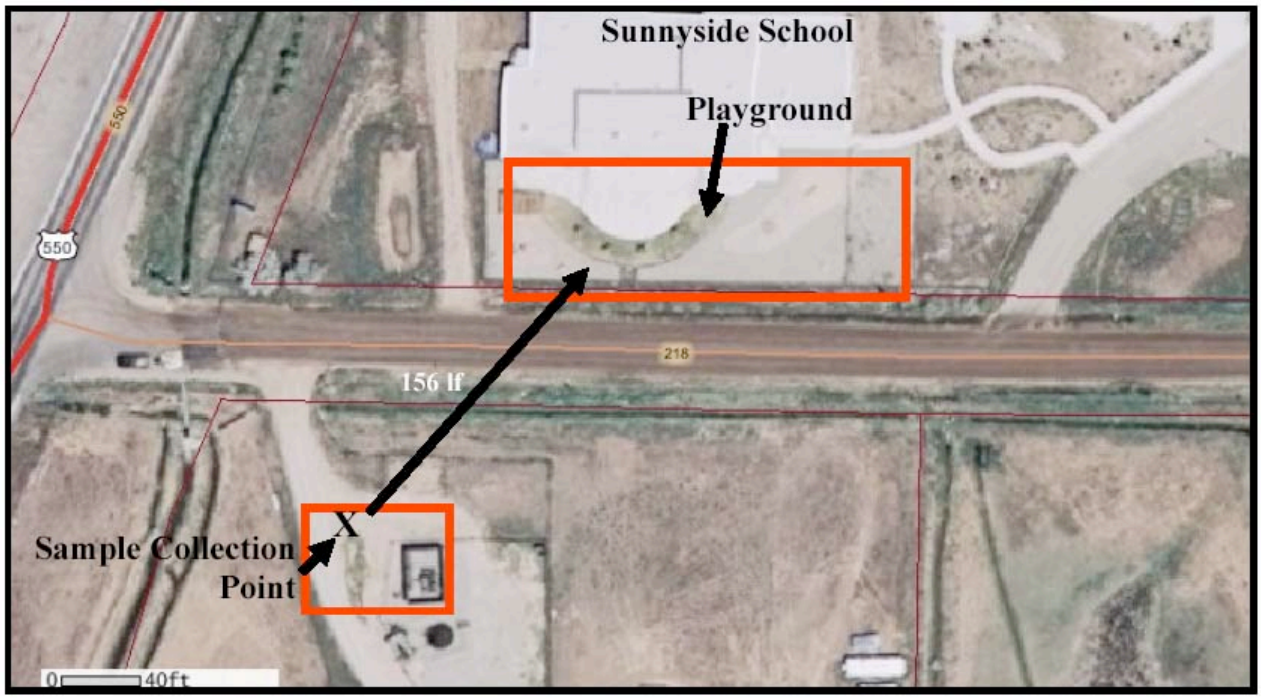
  Long-term exposure to hydrogen sulfide is associated with an elevated incidence of respiratory infections, irritation of the eye and nose, cough, breathlessness, nausea, headache, and mental symptoms, including depression. The World Health Organization's Guideline Value for exposure to hydrogen sulfide is 7 μg/m$^3$ over a 30-minute period.

For the first time, at least two cancer-causing chemicals found at high levels, acrylonitrile and methylene chloride,[20] were detected by the air samples at a variety of natural gas development sites. Neither is associated with natural gas and oil deposits, but both have been shown to be associated with chemicals used in the fracking process to increase yields from oil and gas deposits.

The air samples found high levels of chemicals that can cause symptoms that match the odors and health effects reported by nearby residents for years. This confirms the need for agencies to take such complaints seriously and to better monitor and require pollution controls at all points of natural gas production and processing.

[20] Cherniak, Mark. Data Interpretation Synthesis Letter. 16 Feb 2011

*Image from San Juan Basin Health Department*




**Bucket Brigade Sample Collection Event**
**January 7, 2011**
**Dehydrator Facility Located**
**156 lf from Sunnyside Kindergarten Playground**

**Results near the Sunnyside School in La Plata County, Colorado**

On January 7, 2011, two members of the Bucket Brigade team in La Plata County, Colorado, took an air sample less than 50 feet from a dehydrator that is less than 200 feet from the Sunnyside Elementary School playground near Durango. This natural gas dehydrator is a frequently suspected source of unknown chemical odors. The sampling team on site experienced odors. Subsequent analysis of the air sample revealed a number of toxic chemicals, including four known carcinogens.

A significant level of acrylonitrile, a human carcinogen, was detected in this sample (as it was in four other samples in this report) at a level above which is considered by the US EPA to be an unacceptable long-term exposure risk.[21] Methylene chloride, a human carcinogen, was also detected in this sample (as it was in four other samples) at a level above which is considered to be an unacceptable long-term exposure risk.

Two more carcinogenic substances, benzene and ethylbenzene, were also detected in this sample at levels above that which is considered to be an unacceptable long-term exposure risk. The

[21] http://www.epa.gov/ttn/atw/toxsource/table1.pdf

level of benzene in this sample, 47 ug/m$^3$, is notable in that it is the highest level of benzene detected so far in this area by the Bucket Brigade. Besides acting as a carcinogen, benzene can also adversely impact the human immune system by decreasing circulating levels of lymphocytes. To prevent reduced lymphocyte counts, the U.S. EPA has an established a reference (long-term) concentration for benzene of only 30 ug/m$^3$.

Mark Chernaik, PhD, interpreted the test results for this project. According to Dr. Chernaik, "The level of benzene in this sample is more than 50% above the U.S. Reference concentration for benzene. If this detected level of benzene in this sample represents ambient air quality that generally prevails at this location, then persons living or attending school at this location would be at risk to adverse impacts to the immune system."[22]

The levels of other aromatics in the sample, – 4-ethyltoluene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, – although not above health reference levels are strikingly similar to the levels of these aromatics in four other samples and seem to be a fingerprint for volatile organic compounds near an oil and gas facility in this area. The high levels of the tentatively identified compounds propane and butane also strongly suggest that the source of the volatile organic compounds is related to gas field activities.

**Matching odors and health effects to sample results**

Residents of natural gas production facilities involved in the Bucket Brigade air-testing project recorded their observations and health effects during testing. Once sample results were available, the observed odors and health effects noted in pollution logs were compared to the known health effects of the toxic chemicals found in the samples. Here are several examples:

> *"On Wednesday, Jan 19th air sample was taken at the Blanco, NM Enterprise Buena Vista Compressor Station in Pump Canyon north of several homes. Chris Velasquez and his family live "down wind" of this site. Chris was my guide and companion on the testing trip.*
>
> *I smelled the heavy smell of oily burning plastic. My eyes burned and my nose, throat and mouth became irritated instantly. The soft tissue in my nose, throat and mouth are still sore today as I write this. I have been coughing and my nose is still runny. My eyes are still very red and irritated."*[23]

The sample results confirmed the presence of several noxious benzene compounds, including chlorobenzene, 1, 2, 4-trimethylbenzene and xylene compounds. They are significant irritants to the respiratory system and combined exposure to these could have resulted in the health effects experienced by the sampler.

> *"Warren & I noted additional sharp natural gas/petroleum odors coming from the direction of the BP/CP wells when we did the air sample on January 18th. Warren noted that his eyes were burning. My throat was very irritated and my eyes burned. The musty*

[22] Chernaik, Mark. 25 Jan. 2011
[23] McNall, Shirley. 20 Jan. 2011

> *sewage/feed lot odor is nauseating and causes throat irritation and burning eyes for me.*
>
> *The wells are on BLM land that was granted to the City of Aztec in 1963 for Recreation & Public Use Purposes (R&PP). Some of that land has been granted to Aztec Schools for the new athletic fields and sports complex located about 800 ft. from the wells."*[24]

Sample results from the January 18th sample confirmed the presence of 22 different toxic gases, including cancer-causing benzene. Many of the gases present in the sample irritate the respiratory system and the eyes. Again, the observations recorded by Bucket Brigade air samplers match the sample results.

[24] McNall, Shirley. 20 Jan. 2011

## Recommendations

Given the close proximity of residential and public property, any new sites – whether drilling, fracking, refining, or disposal – should be located at least one-quarter mile from homes, farms, schools, playgrounds, and businesses. This space would provide a **buffer zone** for industry to continue its operations while reducing community exposure to chemical contaminants.

**U.S. EPA** should update air quality standards for oil and gas development, including the New Source Performance Standards (NSPS) and National Emission Standards for Hazardous Air Pollutants (NESHAP), based on the following principles:

Comprehensiveness: All sources of air pollution in the oil and gas sector, including exploration, production, processing, transmission, distribution, and storage, and all pollutants released by these sources should be included in any updated air quality regulations, regardless of the level of emissions or major or area source status.

Air monitoring and transparency: Natural gas development sites including well pads, compressors, gas plants, and waste sites should be required to continuously monitor for volatile organic compounds and hydrogen sulfide in order to ensure compliance with regulations, emission limits and public health protections. All data should be publicly available via the web to provide full transparency to the public.

Effectiveness: The EPA should require the best available control technology and require practices and technologies that both reduce air pollution and promote more efficient oil and gas operations.

Full Health Protection: The EPA should consider prohibiting hazardous air pollutant emissions in certain circumstances, and ensure that any residual risk standards reduce lifetime cancer risk from oil and gas operations to below one in one million.

Forward Looking: EPA should develop mechanisms to ensure that any new equipment, facilities, technologies or practices will be subject to air pollution control requirements that may be required under any updated NSPS and NESHAP.

Enforceability: Any standards should be practicably enforceable by including monitoring, record keeping, and reporting requirements necessary to ensure continuous compliance with the standards and to allow the public, States, and the EPA to easily determine deviations and enforce any noncompliance.

**CONGRESS** should close the gaping loophole in the Clean Air Act's National Emission Standards for Hazardous Air Pollutants (NESHAPs). Oil and gas exploration and production operations are exempt from two key provisions of the NESHAPs, designed to protect public health, allowing the industry to avoid complying with standards that are applied to other industries.

**STATES:**

In 2009, EPA Administrator Lisa Jackson issued a ruling on a Title V petition holding that states must assess whether oil and natural gas operations should be aggregated in accordance with longstanding EPA policies governing New Source Review and Title V permitting.[25] States should follow the EPA's recent guidance and ensure that emissions from oil and gas operations are appropriately aggregated to ensure compliance with New Source Review and Title V. Aggregation provides an important opportunity to more accurately recognize integrated source operations under the Clean Air Act and ensure that oil and gas operations are regulated on a cumulative basis under New Source Review and Title V.

Until strong new rules are in place, the oil and natural gas industry can and should **voluntarily invest in equipment that reduces pollution escaping to the air**. Such equipment is readily available and financially profitable for companies. These investments would increase efficiency and production and reduce cancer causing chemicals from being emitted into the air in communities near production facilities –saving lives and protecting the health of neighboring families.

As the natural gas industry continues to grow, so will the number of families neighboring and affected by the emissions. Industry and government leaders have a unique opportunity to address public health and environmental issues by implementing these recommendations. For coexistence between communities and industry to be possible, chemical exposure has to be immediately addressed.

[25] http://www.epa.gov/region7/air/title5/t5memos/oilgaswithdrawal.pdf

# Pennsylvania Energy Impacts Assessment

*Report 1: Marcellus Shale Natural Gas and Wind*

# Pennsylvania Energy Impacts Assessment

## *Report 1: Marcellus Shale Natural Gas and Wind*

November 15, 2010

Author and Team Leader: Nels Johnson[1]

Assessment Team: Tamara Gagnolet[1], Rachel Ralls[1], Ephraim Zimmerman[2], Brad Eichelberger[2], Chris Tracey[2], Ginny Kreitler[3], Stephanie Orndorff[3], Jim Tomlinson[3], Scott Bearer[1], and Sarah Sargent[3]

The Nature Conservancy gratefully acknowledges generous financial support for this assessment from the Heinz Endowments, the R.K. Mellon Foundation, and the William Penn Foundation.

1. The Nature Conservancy – Pennsylvania Chapter
2. Western Pennsylvania Conservancy – Pennsylvania Natural Heritage Program
3. Audubon Pennsylvania

Cover photos: Marcellus gas drilling rig in Lycoming County © Tamara Gagnolet / TNC; wind turbine in Tioga County © Nels Johnson / TNC; log pile © TNC; electric transmission lines in Clinton County © George C. Gress / TNC

# Table of Contents

Executive Summary ..... 3

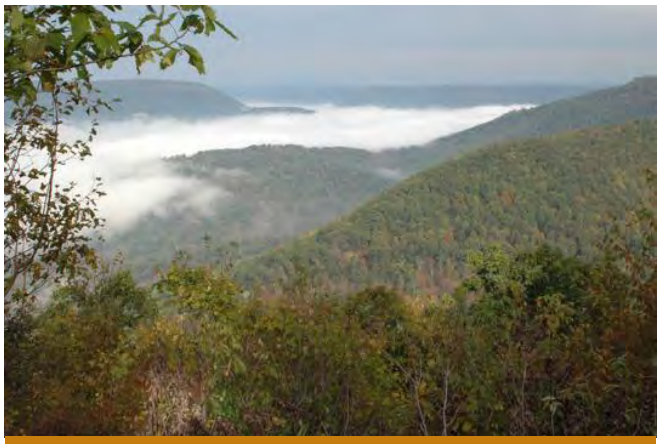
Marcellus Shale Natural Gas ..... 8

- What is Marcellus Shale Natural Gas? ..... 8
- Current and Projected Marcellus Shale Natural Gas Development ..... 9
- Conservation Impacts of Marcellus Shale Natural Gas Development ..... 18
- Key Findings ..... 29
- Additional Information ..... 30

Wind ..... 31

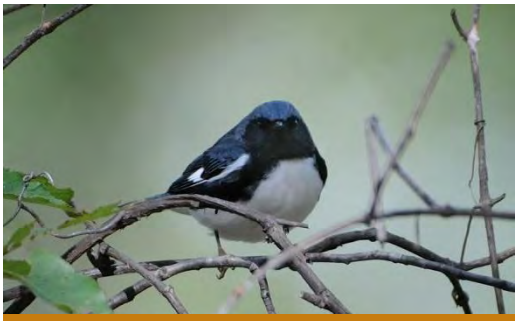
- What is Wind Energy? ..... 31
- Current and Projected Wind Energy Development ..... 32
- Conservation Impacts of Wind Energy Development ..... 38
- Key Findings ..... 44
- Additional Information ..... 45

# Executive Summary



Forest landscape along the West Branch Susquehanna River, Clinton County. © George C. Gress / TNC

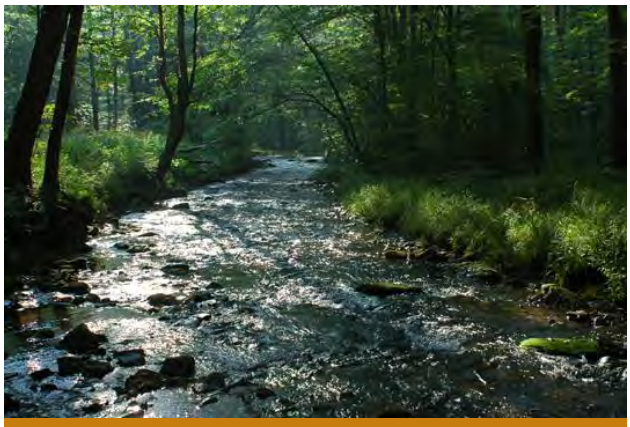
Within a few weeks during the summer of 2000, eight towers rose two hundred feet above an agricultural field on a low ridge top along the Pennsylvania Turnpike. Not long after, large blades began sweeping the Somerset County sky as Pennsylvania's first industrial wind facility went on line. Several years later and an hour drive to the west, an unusual natural gas well was drilled over a mile down and pumped full of water. That well in Washington County yielded a surprising amount of gas flowing from fractures in a shale formation that geologists had long suspected held plenty of gas but has been too expensive to develop. Meanwhile, a Canadian company bought a small sawmill in Mifflintown and started producing wood pellets for stoves, boilers, and electric plants. It soon became one of the region's largest producers of wood biomass energy supplies. In the decade since, these three new energy technologies have expanded rapidly across the state. By the end of this year, 500 wind turbines will be turning on Pennsylvania ridgelines, nearly 2,000 Marcellus natural gas wells will be scattered across rolling fields and forests, and over 50 facilities will be producing wood pellets or burning wood for energy. Thousands of miles of pipelines and powerlines already crisscross the state to get energy supplies to major markets in the Northeast.



Black-throated blue warblers and other interior forest species could be impacted by forest fragmentation caused by energy development. © Gary Irwin

Each of these energy sources carries both promise and risk for people and nature. The promise is that wind, natural gas, and wood biomass energy can reduce greenhouse gas emissions, generate jobs, and increase energy security. The risk is that extensive land use change and loss of natural habitats could accompany new energy development and transmission lines. Impacts to priority conservation habitats across the state have been modest thus far. For example, aerial photo analysis indicates Marcellus gas development has so far cleared just 3,500 acres of forest (about 1,000 acres for wind turbines). An additional 8,500 acres of forest is now within 300 feet of new fragmenting edges created by well pads, and associated roads and infrastructure (5,000 acres for wind turbines). This fragmentation deprives "interior" forest species, such as black-throated blue warblers, northern goshawks, salamanders, and many woodland flowers, of the shade, humidity and tree canopy protection that only deep forest environments can provide.

By all accounts, each of these energy types is likely to grow substantially in Pennsylvania during the next two decades. The Marcellus shale formation, which underlies two-thirds of the state, is now believed to be one of the largest unconventional shale gas reserves in the world. The Pennsylvania Alternative Energy Portfolio Standards Act of 2004, along with state and federal incentives, will likely boost expansion of wind, wood biomass, and other alternative energy types over the next two decades. But, how much of each energy type might be developed? What transmission infrastructure will be needed to get more electric power and natural gas to consumers? And, where are these energy types most likely to be developed? How does the likely scale and location of future energy development overlap with priority conservation areas? The Pennsylvania Energy Impacts Assessment seeks answers to these questions so that conservationists can work more effectively with energy companies and government agencies to avoid, minimize or mitigate habitat impacts in the future.



Nine Mile Run Creek in PA's North Central Highlands
© George C. Gress / TNC.

**Assessment Goal:** Develop credible energy development projections and assess how they might affect high priority conservation areas across Pennsylvania. Marcellus natural gas, wind, wood biomass, and associated electric and gas transmission lines were chosen as the focus since these energy types have the most potential to cause land-use change in the state over the next two decades. The conservation impacts focus is on forest, freshwater, and rare species habitats. The assessment **does not** address other potential environmental impacts, including water withdrawal, water quality, air quality and migratory pathways for birds and bats. The assessment also does not address a range of other social, economic, and climate characteristics of these energy types.

**Key Assumptions:** Any assessment of future trends must include certain assumptions. Among the most important assumptions of the Pennsylvania Energy Impacts Assessment are the following:

- A 20-year time period is used to assess potential cumulative habitat impacts from energy development;
- Given uncertainties about how energy prices could change, it was assumed that prices and capital investment (and policy and social conditions) will be sufficient to promote steady development growth for each energy type during the next two decades;
- Given uncertainty about how technology changes could affect spatial footprints, it was assumed that spatial footprints per well pad, turbine, and mile of transmission line will not change significantly during the next two decades;
- Given the proprietary nature of data on leases, Marcellus Shale porosity, fine resolution wind power, etc., all projections are based on publicly available information;
- It was assumed that recent trends and patterns of energy development will continue for the next two decades absent significant changes in government policies and industry practices;

Energy projections contained in this assessment are informed scenarios – **not predictions** – for how much energy development might take place and where it is more and less probable. Projected impacts, however, are based on measurements of actual spatial footprints measured for hundreds of well pads and wind turbines.

**Analytical Steps:** Key analytical steps for the Pennsylvania Energy Assessment included:

1) *Data collection* – Over 50 spatial data layers on energy resources, development permits, road and transmission infrastructure, physical features, and conservation priorities were compiled for the assessment;

2) *Spatial footprint analysis* – Spatial footprints for Marcellus gas well and wind turbine pads, associated roads, associated pipelines, associated electric transmission lines, and associated other clearings (*e.g.*, gas containment pits, equipment staging areas, electrical substations) were digitized using aerial photos of sites before and after construction;

3) *Scale projections* – Low, medium, and high scenarios for ***how much*** Marcellus Shale natural gas, wind, wood biomass, and transmission line development might occur were based as much as possible on existing projections and data from credible sources.

4) *Geographic projections* – Projections of ***where*** new Marcellus natural gas and wind energy development is more and less likely to occur were based on modeling the probability of a map pixel's land-use change to energy production based on sets of drivers and constraints developed for each energy type. Geographic projections for wood biomass and energy transmission were not modeled due to a lack of data. Conclusions about regional patterns of wood biomass and transmission development and potential conservation impacts will be presented in Report 2 of the Pennsylvania Energy Impacts Assessment.

5) *Conservation impacts analysis* – The potential impacts of future energy development were assessed for forest and freshwater habitats across the state. In addition, sites recognized as important for species of conservation concern were assessed. Conservation datasets for these assessments included, among others, large forest patches from The Nature Conservancy and the Western Pennsylvania Conservancy, habitat areas for rare species from the Pennsylvania Natural Heritage Program, densities for interior forest nesting bird species from the 2nd Pennsylvania Breeding Bird Atlas, and intact watersheds for native brook trout populations from the Eastern Brook Trout Joint Venture.

6) *Review* – A dozen energy experts in government, industry, and research organizations provided technical review of the energy projections.

**Energy Projections:** The Pennsylvania Energy Impacts Assessment developed low, medium and high scenarios for the amount of energy development that might take place in Pennsylvania by 2030. The projections include:

- *Marcellus Shale* – Sixty thousand wells could be drilled on between 6,000 and 15,000 new well pads (there are currently about 1,000), depending on how many wells are placed on each pad. Gas development will occur in at least half of the state's counties, with the densest development likely in 15 counties in southwest, north central, and northeast Pennsylvania.

- *Wind* – Between 750 and 2,900 additional wind turbines could be built (there are currently about 500), depending on the wind share of electric generation by 2030. Most turbines would be built along the Allegheny Front in western Pennsylvania and on high Appalachian ridgetops in the central and northeastern parts of the state.

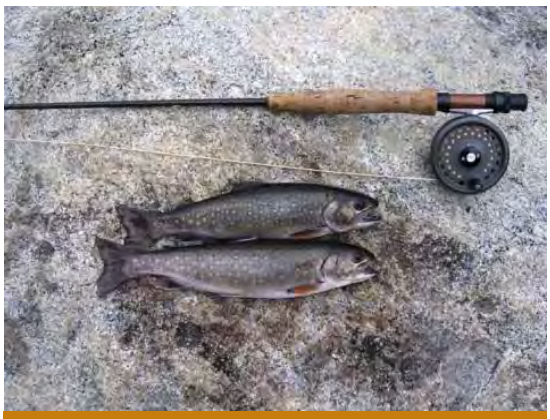
- *Wood Biomass* – Wood biomass energy demand could double or even triple today's wood energy use, depending on whether and how many coal power plants co-fire with wood biomass. Wood biomass energy development is likely to be widespread across the state in all three scenarios.

- *Transmission Lines* – Preliminary findings indicate between 10,000 and 15,000 miles of new high-voltage power lines and gas pipelines (especially gathering lines) could be built during the next twenty years. There is considerable uncertainty about exactly where these lines will be built but recently proposed electric and gas transmission lines provide insights into potential habitat impacts.

**Conservation Impacts:** This first Pennsylvania Energy Impacts Assessment report focuses on the overlap between likely Marcellus gas and wind development areas and Pennsylvania's most important natural habitats. A second report will focus on the potential for additional impacts from new wood biomass energy plants, electric power lines, and natural gas pipelines. Key findings for impacts from Marcellus natural gas and for wind development include:

***Forests***. By 2030, a range of between 38,000 to 90,000 acres of forest cover could be cleared by new Marcellus gas development in the state. Forest clearing for the wind development scenarios is much smaller, ranging from 1,900 to 5,200 acres. Such clearings would create new forest edges where the risk of predation, changes in light and humidity levels, and expanded presence of invasive species could threaten forest interior species in 91,000 to 220,000 forest acres adjacent to Marcellus development and 13,400 to 36,000 forest acres adjacent to wind development. Forest impacts will be concentrated in the north central and southwest parts of the state where many of the state's largest and most intact forest patches could be fragmented into smaller patches by well pads, roads, and other infrastructure. Impacts to forest interior species will vary depending on their geographic distribution and density. Some species, such as the black-throated blue warbler, could see widespread impacts to their relatively restricted breeding habitats in the state while widely distributed species, such as the Scarlet Tanager, would be relatively less affected. Locating energy infrastructure in open areas or toward the outer edges of large patches can significantly reduce impacts to important forest areas.

***Freshwater.*** Aquatic habitats are at risk too. Once widespread, healthy populations of native eastern brook trout in Pennsylvania are now largely confined to small mountain watersheds. Nearly 80 percent of the state's most intact brook trout watersheds could see at least some Marcellus gas and wind development during the next twenty years. Strongholds for brook trout are concentrated in north central Pennsylvania, where Marcellus development is projected to be relatively intensive in over half of the state's best brook trout watersheds. Exceptional Value streams – the Department of Environmental Protection's highest quality designation – could see hundreds of well pads (perhaps 300 - 750) and dozens of wind turbines (perhaps 50 – 200) located within one-half mile under the projections. Because many intact brook trout



Brook trout © TNC

and EV streams are in steep terrain, rigorous sediment controls, and possibly additional setback measures, are needed to help conserve these sensitive habitats.

***Rare Species***. Nearly 40 percent of Pennsylvania's globally rare and Pennsylvania threatened species can be found in areas with high potential for Marcellus gas development. These species tend to be associated with riparian areas, streams, and wetlands, while others are concentrated in unusually diverse areas such as the Youghiogheny Gorge. A handful of rare species have most or all of their known locations in high potential areas for Marcellus gas development. For example, three-fourths of all known snow trillium populations are in high potential Marcellus development areas as are all known populations for the green salamander. A much smaller number of known locations for globally and state rare species overlap with high potential wind development sites and they tend to be associated with rocky outcrops and ridgetop barrens habitats. Species with the greatest overlaps include timber rattlesnakes, Allegheny woodrats, and northern long-eared Myotis bats. More intensive surveys for globally rare and state critically endangered species in high potential Marcellus and wind development areas could help to minimize impacts before development begins. The Pennsylvania Game Commission is working with wind companies and other researchers to assess impacts to migratory pathways for birds and bats.

***Recreation.*** Extensive overlaps are projected between Marcellus development and state forests, state parks, and state game lands. Just over ten percent of Pennsylvania's public lands are legally protected from gas development, most of it within State Wild and Natural Areas or in state parks where the Commonwealth owns the mineral rights. The state does not own mineral rights for 80% of State Park and State Game Lands, nearly 700,000 acres of State Forests have already been leased, and only about 300,000 acres of the remaining State Forest Lands are legally off-limits to future leases. Projections indicate between 900 and 2,200 well pads could be developed across all state lands, with most going on State Forest Lands, followed by State Game Lands, and State Parks. Wind development was not projected on state lands, though some facilities are projected near highly visited sites, including natural vistas.

Clearly, the heart of some of Pennsylvania's best natural habitats lies directly in the path of future energy development. Integrating information on conservation priorities into energy planning, operations, and policy by energy companies and government agencies sooner rather than later could dramatically reduce these impacts. Many factors – including energy prices, economic benefits, greenhouse gas reductions, and energy independence – will go into final decisions about where and how to proceed with energy development. Information about Pennsylvania's most important natural habitats should be an important part of the calculus about trade-offs and optimization as energy development proceeds. Would Pennsylvania's conservation pioneers, including Gifford Pinchot, Maurice Goddard, and Rachel Carson, expect anything less?

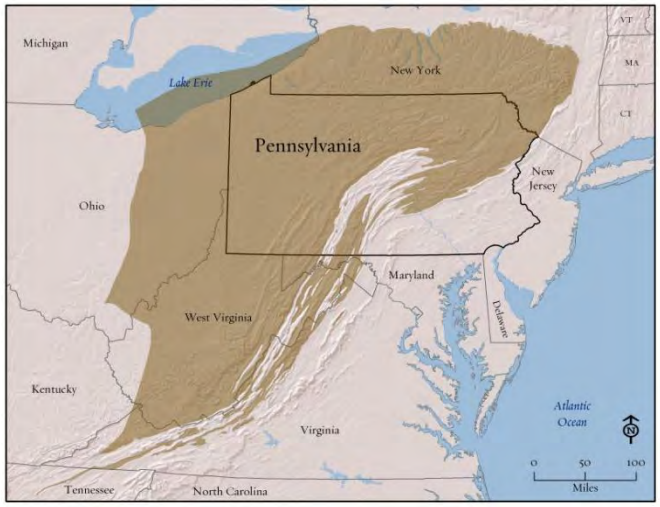
# Marcellus Shale Natural Gas

Once thought to be inaccessible, deep shale formations with tightly held natural gas have become the most rapidly growing source of energy in North America. New technologies and methods have allowed companies to drill 6,000 to 10,000 feet down to reach the Marcellus shale, turn the well horizontally to follow the shale layer for a mile or more, and then pump in millions of gallons of water to fracture the shale and release the natural gas. Pennsylvania is at the epicenter of the Marcellus formation, one of the world's largest unconventional shale natural gas reserves. Situated right next door to huge markets in the Mid-Atlantic and Northeastern states, Marcellus gas development has expanded at a furious pace since the first wells were drilled just few years ago in Washington County. There are now approximately 2,000 drilled wells, most of them concentrated in the southwestern and northeastern parts of the state.

The Marcellus boom is bringing rapid economic growth to many rural communities that have been in economic decline for decades. Natural gas is also displacing higher carbon coal and oil supplies thus slowing the rise in greenhouse gas emissions. These benefits are real but not without costs. Large amounts of water must be withdrawn to frac each well (about 5 million gallons). The return flow water that comes back up from the well contains varying levels of chemicals, heavy metals, and even radioactive materials, and must be handled carefully to avoid spills when recycled or disposed. Heavy trucks and compressor stations rumble constantly in gas development areas putting heavy strains on roads, bridges and air quality. Because of known and perceived risks to environmental quality and human health, water use, air emissions and transportation demands are receiving growing attention from government agencies, researchers and energy companies. Thus far, relatively little attention, however, has been focused on Marcellus gas development impacts to natural habitats across the state.

## What is Marcellus Shale Natural Gas?

The Marcellus is the largest gas-bearing shale formation in North America in both area and potential gas volume. It spans over 150,000 square miles across 5 states including the southern tier of New York, the northern and western half of Pennsylvania, the eastern third of Ohio, most of West Virginia, and a small slice of western Virginia. Estimates of the potential recoverable volume have increased steadily. The latest estimates by the U.S. Department of Energy are nearly 300 trillion cubic feet – enough to supply all natural gas demand in the United States for at least 10 years.



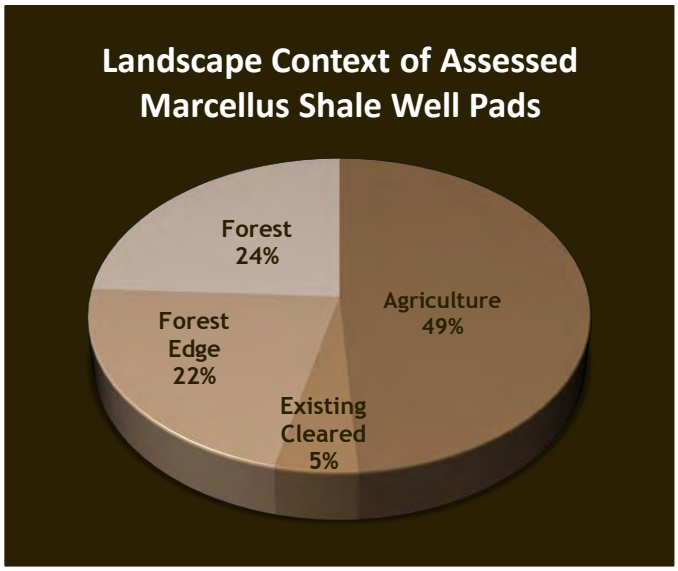

Map showing the extent of the Marcellus Shale formation. Data source: United States Geological Survey.

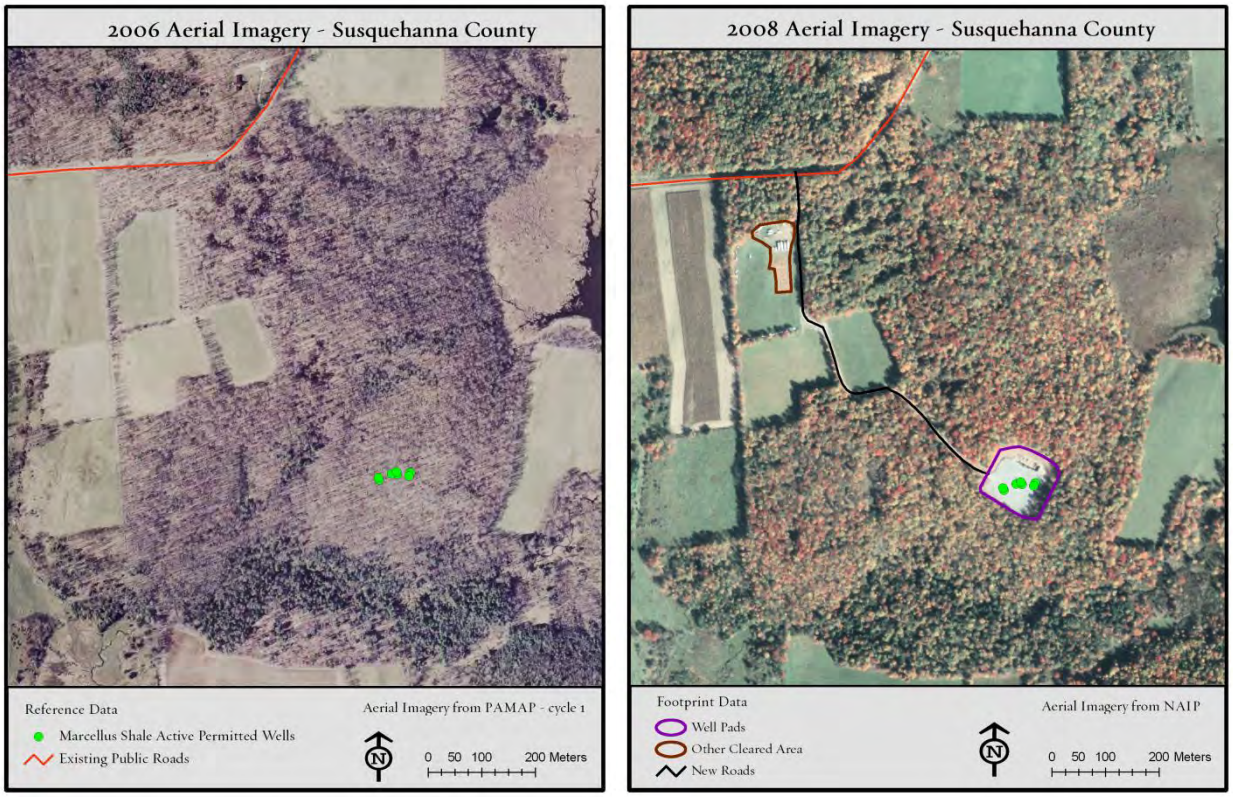
Geologists have long known the Marcellus formation is an organically-rich shale with potentially large amounts of natural gas, but it was too deep, too thin, and too dense to exploit. In 2005, Range Resources drilled the first production Marcellus well using horizontal drilling and hydraulic fracturing methods. The horizontal drilling is necessary because the shale is typically thin and vertical wells will only intercept a small part of the formation. Hydraulic fracturing (or "fracing") is a process that uses large volumes of water, sand, lubricants, and other chemicals to create small fissures in the shale rock. Hydro-fracing is necessary to release the gas which is tightly held in the dense black shale. These methods, first perfected for deep shale gas in the Barnett formation of Texas, unlocked the tremendous gas reserves in the Marcellus and other "unconventional" shale formations previously thought to be out of economic reach.

In contrast to shallow gas deposits in western Pennsylvania, the Marcellus is developed with multiple horizontal wells that can reach out 5,000 feet or more from one well pad. Everything about Marcellus development is bigger than conventional shallow gas plays. The well pads are more expansive (averaging just over 3 acres compared to a small fraction of an acre), the water used to frac wells is much greater (5 million gallons versus a hundred thousand gallons), and the supporting infrastructure is much larger in scale (24" diameter pipelines to gather gas from wells versus 2" or 4" pipelines in shallow fields). Individual wells are also vastly more productive (5 – 10 million cubic feet per day versus less than 100,000 cubic feet in peak early production). While the larger pad, greater water use, and more extensive infrastructure pose more challenges for conservation than shallow gas, the area "drained" by wells on each Marcellus pad is much larger than from shallow gas pads (500-1,000 acres versus 10-80 acres) since there are typically multiple lateral wells on a Marcellus pad versus a single vertical well on a shallow gas pad. The lateral reach of Marcellus wells means there is more flexibility in where pads and infrastructure can be placed relative to shallow gas. This increased flexibility in placing Marcellus infrastructure can be used to avoid or minimize impacts to natural habitats in comparison to more densely-spaced shallow gas fields.

## Current and Projected Marcellus Shale Natural Gas Development

Projections of future Marcellus gas development impacts depend on robust spatial measurements of existing Marcellus well pads and infrastructure. By comparing aerial photos of Pennsylvania Department of Environmental Protection (DEP) Marcellus well permit locations taken before and after development, we precisely documented the spatial foot print of 242 Marcellus well pads (totaling 435 drilling permits) in Pennsylvania visible in 2008 aerial imagery from the National Agriculture Imagery Program. The ground excavated for wells and associated infrastructure is the most obvious spatial impact. For each well site, areas cleared for the well pad, new or expanded roads, gathering pipelines, and water impoundments were digitized and measured.






Aerial photos before and after development of a Marcellus gas well pad site in Susquehanna County, PA. To assess the impacts of this type of energy development, we digitized the spatial footprint of 242 gas well pad sites and associated infrastructure.

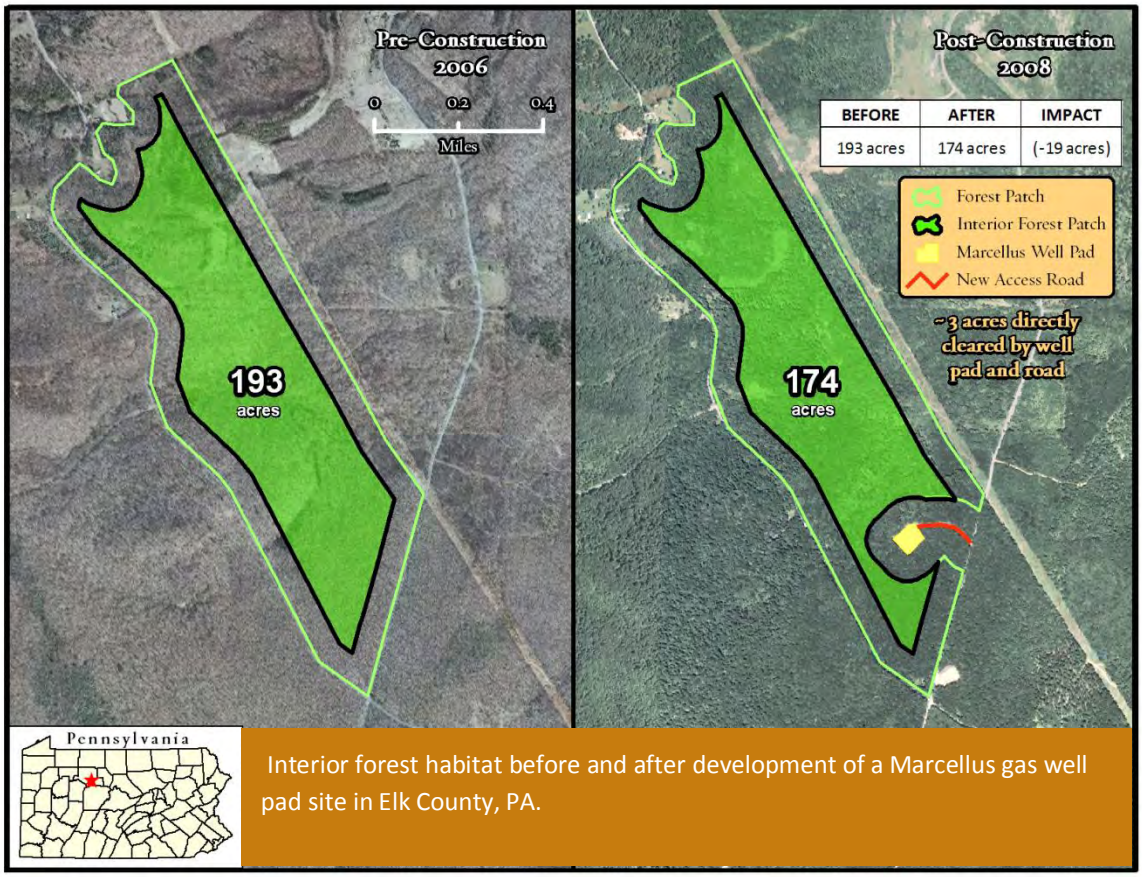
| Average Spatial Disturbance for Marcellus Shale Well Pads in Forested Context (acres) | | |
|---|---|---|
| **Forest cleared for Marcellus Shale well pad** | **3.1** | **8.8** |
| **Forest cleared for associated infrastructure (roads, pipelines, water impoundments, etc.)** | **5.7** | |
| **Indirect forest impact from new edges** | **21.2** | |
| **TOTAL DIRECT AND INDIRECT IMPACTS** | **30** | |

Well pads occupy 3.1 acres on average while the associated infrastructure (roads, water impoundments, pipelines) takes up an additional 5.7 acres, or a total of nearly 9 acres per well pad.

Adjacent lands can also be impacted, even if they are not directly cleared. This is most notable in forest settings where clearings fragment contiguous forest patches, create new edges, and change habitat conditions for sensitive wildlife and plant species that depend on “interior” forest conditions.

Forest ecologists call this the "edge effect." While the effect is somewhat different for each species, research has shown measurable impacts often extend at least 330 feet (100 meters) forest adjacent to an edge. Interior forest species avoid edges for different reasons. Black-throated blue warblers and other interior forest nesting birds, for example, avoid areas near edges because of the increased risk of predation. Tree frogs, flying squirrels and certain woodland flowers are sensitive to forest fragmentation because of changes in canopy cover, humidity and light levels. Some species, especially common species such as whitetail deer and cowbirds, are attracted to forest edges – often resulting in increased competition, predation, parasitism, and herbivory. Invasive plant species, such as tree of heaven, stilt grass, and Japanese barberry, often thrive on forest edges and can displace native forest species. As large forest patches become progressively cut into smaller patches, populations of forest interior species decline.

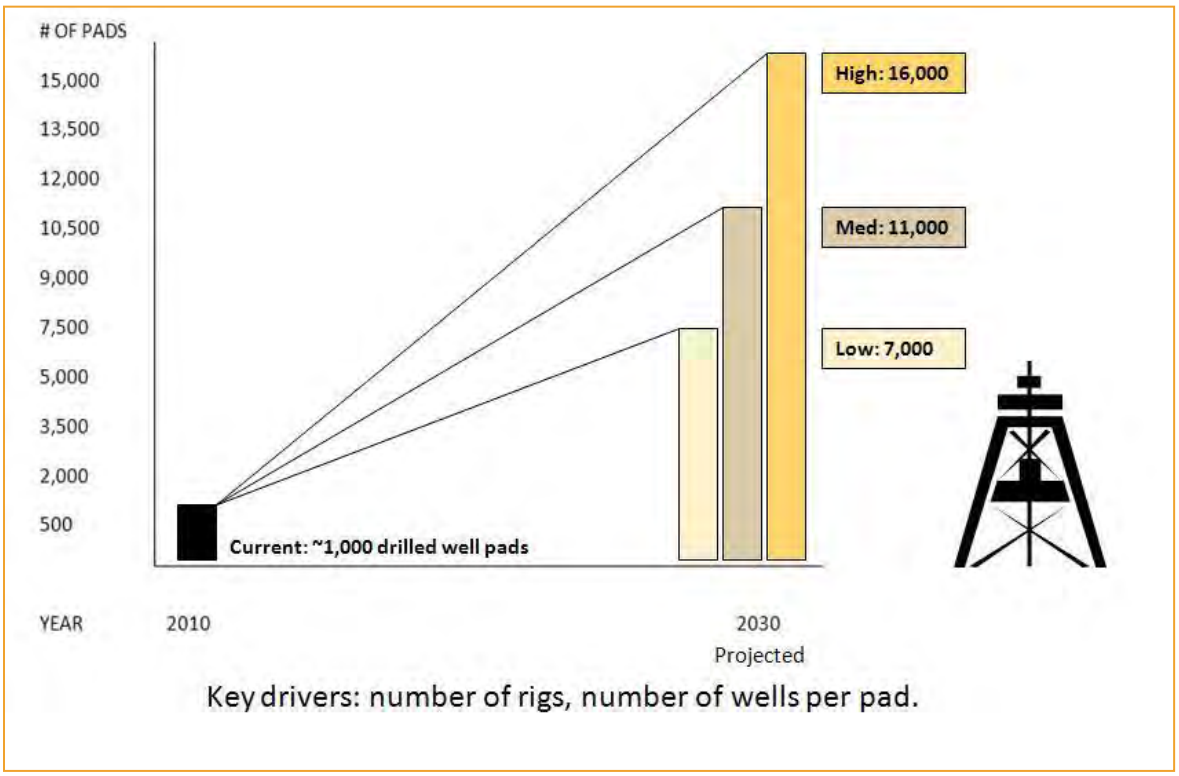
To assess the potential interior forest habitat impact, we created a 100 meter buffer into forest patches from new edges created by well pad and associated infrastructure development. For those well sites developed in forest areas or along forest edges (about half of assessed sites), an average of 21 acres of interior forest habitat was lost.



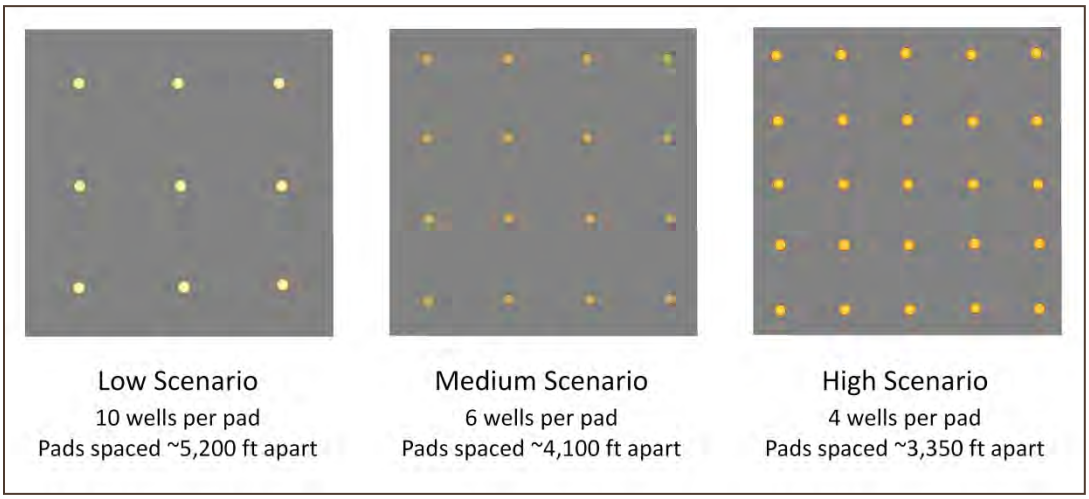

Interior forest habitat before and after development of a Marcellus gas well pad site in Elk County, PA.

The number of Marcellus wells drilled in Pennsylvania during the next two decades will expand steadily. Just how many wells are drilled will be driven by various factors including natural gas prices, technological improvements, human resources, regulatory changes in Pennsylvania and beyond (e.g., end of New York drilling moratorium), and social preferences. Assessing how these factors will change over the next two decades is very difficult; therefore our projections assume economic, policy, and social conditions remain stable enough to promote steady expansion of Marcellus gas development in the state. The first key variable in our projection is the number of drilling rigs that

will be operating in Pennsylvania. By October 2010, the industry had moved just over 100 rigs into Pennsylvania to drill Marcellus wells according to the Baker-Hughes weekly rig count. Given the high productivity of the Marcellus and its proximity to major northeastern markets, most industry observers expect this number to continue growing steadily. The number of horizontal drill rigs operating in the Barnett Shale has peaked at about 200, but the




We project 60,000 Marcellus wells will be drilled during the next twenty years based on company investor presentations and academic assessments of gas development potential. Depending on how many wells on average are placed on the same pad site (see illustration below), we project between 7,000 and 16,000 total well pad sites will be developed in Pennsylvania by 2030.



Marcellus Shale is much larger and could reach 300 rigs in Pennsylvania alone. We chose a conservative estimate of 250 maximum horizontal drill rigs for each scale projection scenario. Assuming that each rig can drill one well per month, 3,000 wells are estimated to be drilled annually. At that rate, 60,000 new wells would be drilled by the year 2030.

The second key variable, especially for determining land-use and habitat impacts, is the number of wells on each pad. Because each horizontal well can drain gas from 80 to 170 acres (depending on the lateral well length), more wells per pad translates to less disturbance and infrastructure on the landscape. It is technically possible to put a dozen or more Marcellus wells on one pad. So far, the average in Pennsylvania is two wells per pad as companies quickly move on to drill other leases to test productivity and to secure as many potentially productive leases as possible (leases typically expire after 5 years if there is no drilling activity). In many cases, the gas company will return to these pads later and drill additional wells. The low scenario (6,000 well pads) assumes that each pad on average will have ten wells. Because many leases are irregularly shaped, in mixed ownership, or the topography and geology impose constraints, it is unlikely this scenario will develop. It would take relatively consolidated leaseholds and few logistical constraints for this scenario to occur. The medium scenario for well pads assumes 6 wells on average will be drilled from each pad, or 10,000 new well pads across the state. Industry staff generally agree that six is the most likely number of wells they will be developing per pad for most of their leaseholds, at least where lease patterns facilitate drilling units of 600 acres or larger. The high scenario assumes each pad will have 4 wells drilled on average, or 15,000 well pads across the state. This scenario is more likely if there is relatively little consolidation of lease holds between companies in the next several years.

The number of well pads is less important than where they are located, at least from a habitat conservation perspective. To understand which areas within Pennsylvania's Marcellus formation are more and less likely to be developed, we used a machine-based learning modeling approach known as maximum entropy (Maxent 3.3.3a, Princeton University). Maximum entropy was used to find relationships between 1,461 existing and permitted well pad locations and variables that might be relevant to a company's decision to drill a Marcellus well. Such variables were chosen based on data availability and included Marcellus Shale depth, thickness and thermal maturity as well as percent slope, distance to pipelines, and distance to roads. The model produces a raster surface that represents the probability of an area to potentially support future gas well development. An additional 487 existing and permitted well pads were used to test the validity of the model's probability surface and the model was found to be 80% accurate in predicting existing and permitted wells from randomly sampled undeveloped areas. The resulting probability map indicates wide variation across the Marcellus formation in terms of the likelihood of future gas well development.

To get a better sense of where gas development is most likely, we searched for the highest probability areas where well pads in each scenario might be located. The probability raster was re-sampled to a resolution that reflects the minimum separation distance between well pads for each of the three impact scenarios (low – 1,590 m; medium – 1,260 m; high – 1,020 m). The minimum separation distance depends on the number of wells drilled per pad and accounts for the average gas drainage area assumed for each of the three scenarios. Areas incompatible for future gas exploration (existing drilled Marcellus Shale wells, Pennsylvania State Wild Areas and Natural Areas, and water bodies) were excluded from being selected as probable pixels. For each scenario, the highest probable pixels were selected until the pad threshold was reached (low – 6,000 well pads; medium – 10,000 well pads; high – 15,000 well pads). The highest probable pixels were then converted into points for map display purposes.

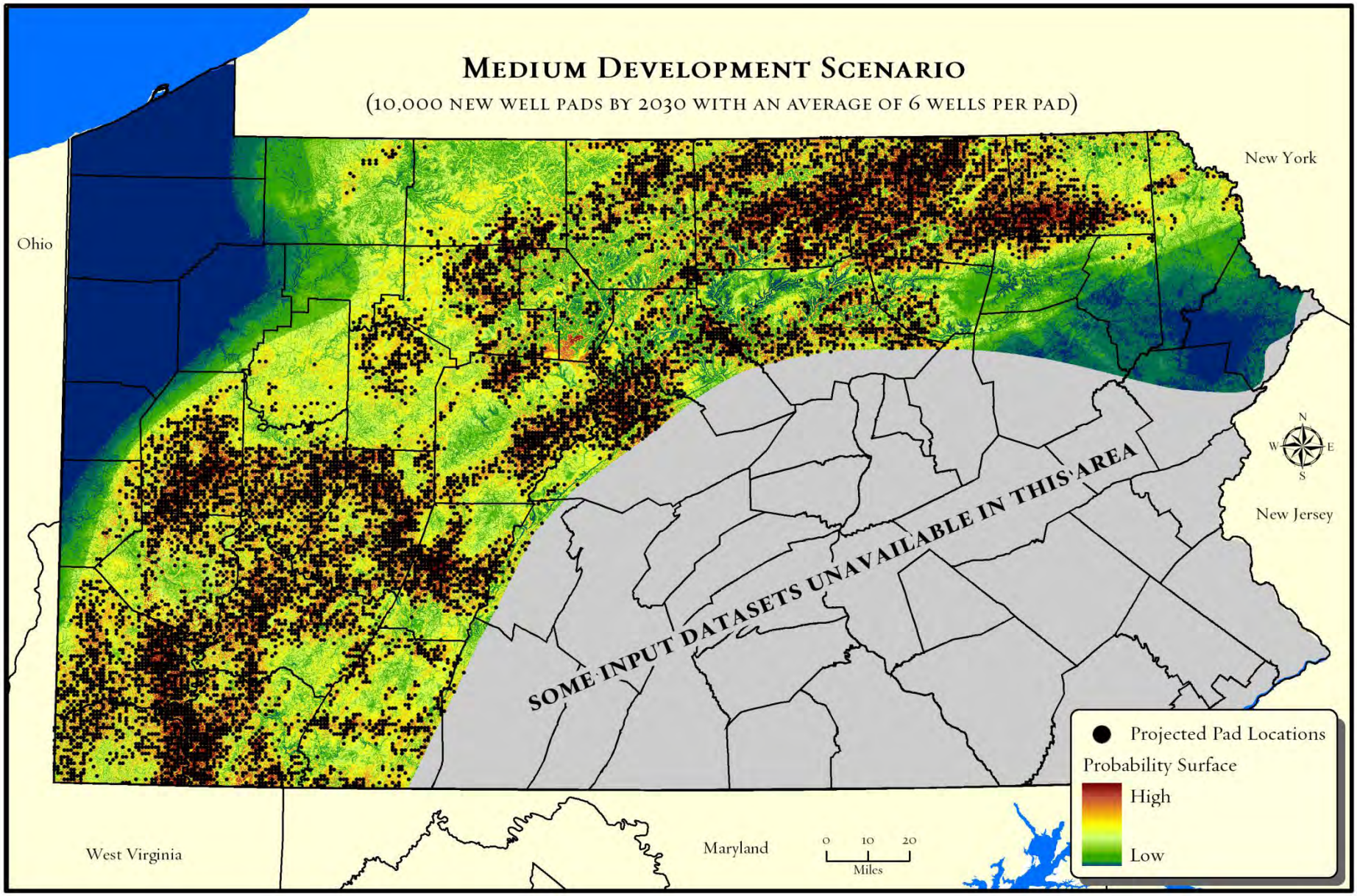
While the geographic area with projected well pads expands from low to high scenarios, the overall geographic pattern is not cumulative due to the differences in minimum separation distance between the three scenarios. Overall, hotspots for future gas development can be seen in half a dozen counties in southwestern Pennsylvania and half a dozen counties in north central and northeastern parts of the state.

These geographic projections of future Marcellus gas development are spatial representations of possible scenarios. They are not predictions. We faced several constraints in developing the geographic scenarios:

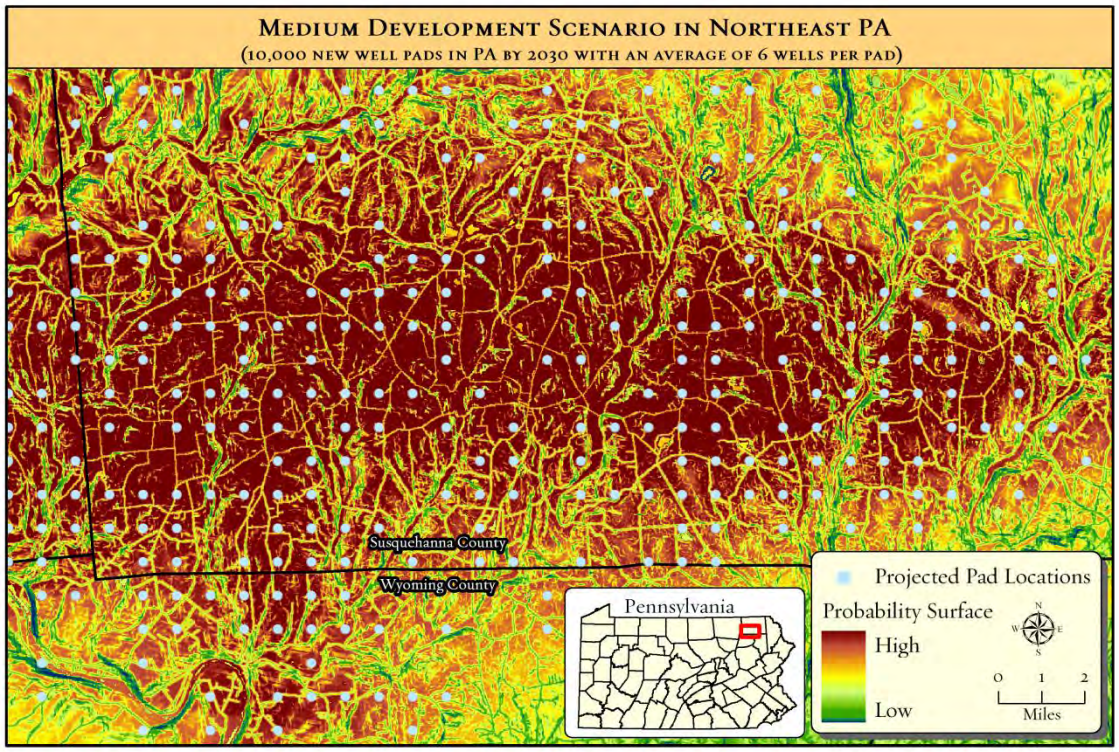
- We do not have access to proprietary seismic and test well geologic data that natural gas companies have. Shale porosity, for example, is a key factor but there are no publicly available data for this.

- We do not have the detailed location of gas company leases. Each company is looking for the highest probability locations across their lease holds while our model looks for the highest probability sites across the entire Marcellus formation in the state. Because there have only been a few Marcellus test wells and permits in the Delaware watershed, we believe the projections for new well pads are probably significantly underestimated in Wayne County.

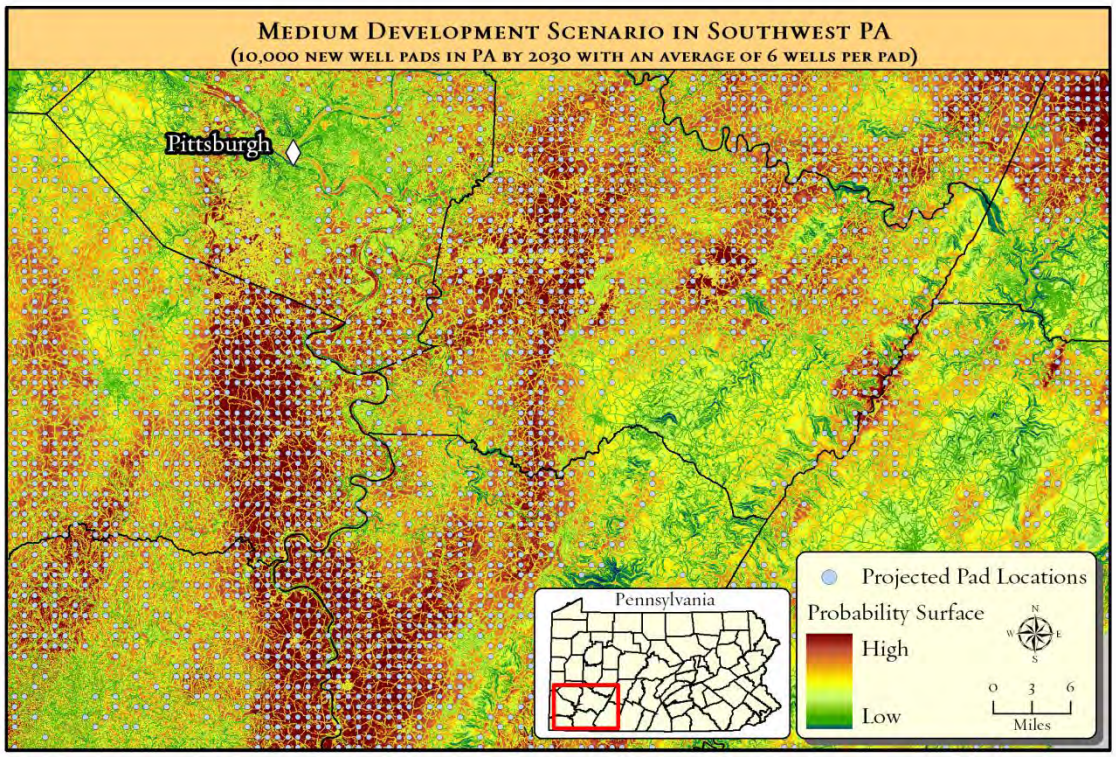
Still, we believe the overall geographic patterns in the projected gas development locations are relatively robust for several reasons. We used nearly 1,500 existing drilled or permitted well pads to build the model and nearly 500 additional drilled and permitted well pads to validate the model. These unique well pad locations represented 4,446 permitted wells. This is typically a sufficient sample size for building predictive models. Additionally, reviews from industry, academic, and government agency reviewers indicate our methods and results are generally sound. Some reviewers expect future well pad locations to be more geographically expansive than our current projections indicate, especially in the Delaware watershed where only a few Marcellus test wells and permits have been issued. Our projections for Wayne County, for example, are likely underestimating future development potential.



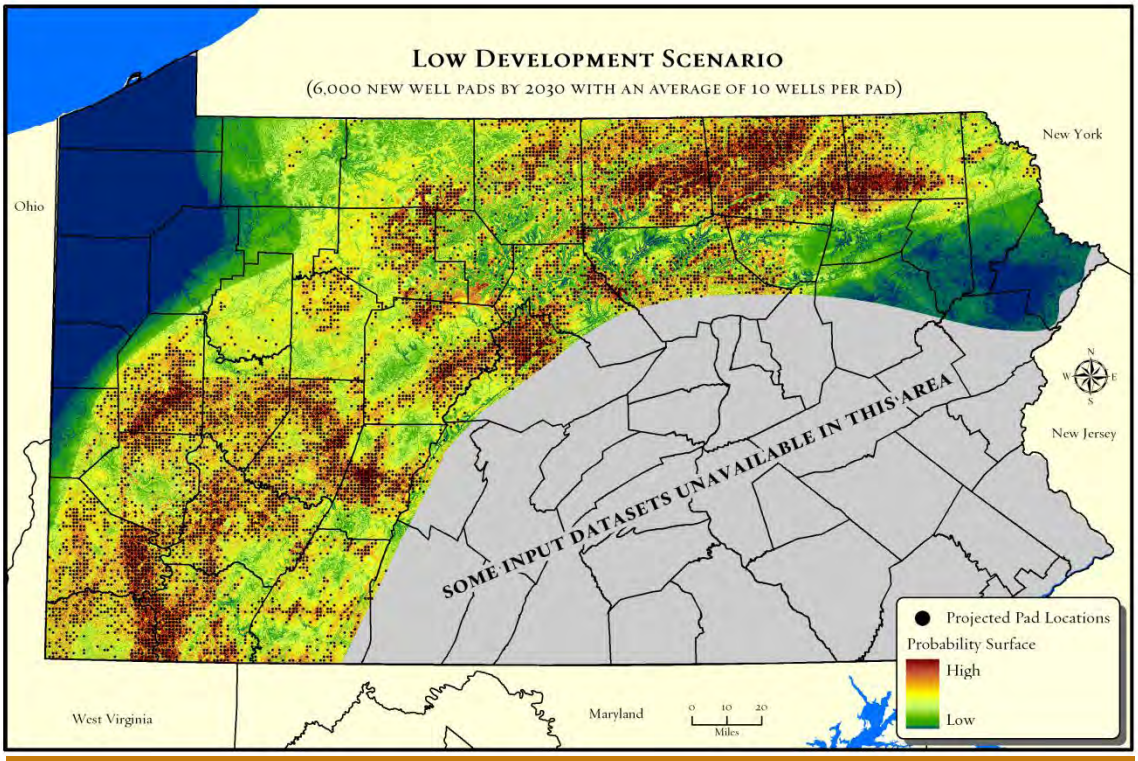

Map showing projected location of 10,000 new Marcellus Shale natural gas pads across Pennsylvania (medium development scenario).



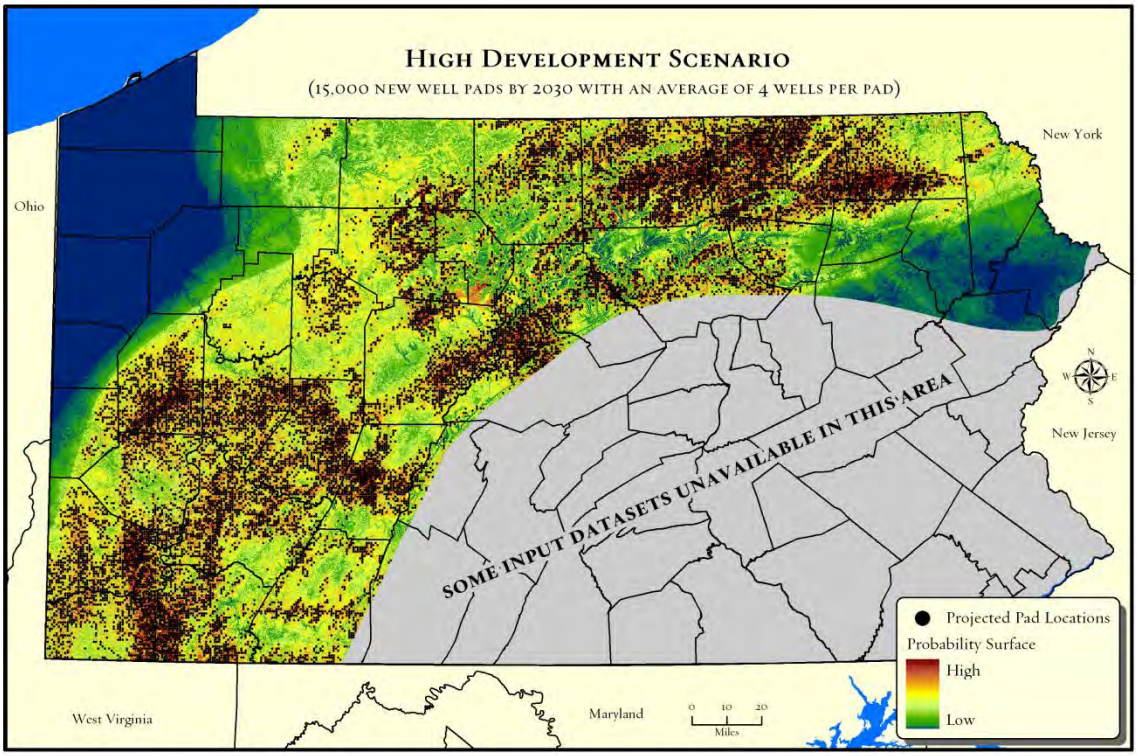

Map showing projected location of new Marcellus well pads in southern Susquehanna County under the medium development scenario.




Map showing projected location of new Marcellus well pads in southwestern Pennsylvania under the medium development scenario.




Map showing projected location of 6,000 new Marcellus well pads across Pennsylvania (low development scenario).




Map showing projected location of 15,000 new Marcellus well pads across Pennsylvania (high development scenario).

## Conservation Impacts of Marcellus Shale Natural Gas Development

What is the overlap of the areas with the highest probability of future Marcellus gas development and those areas known to have high conservation values? To answer this question, we intersected the projected Marcellus well pads with areas previously identified and mapped as having high conservation values. We looked at several examples from four categories of conservation value, including:

- Forest habitats
- Freshwater habitats
- Species of conservation concern
- Outdoor recreation

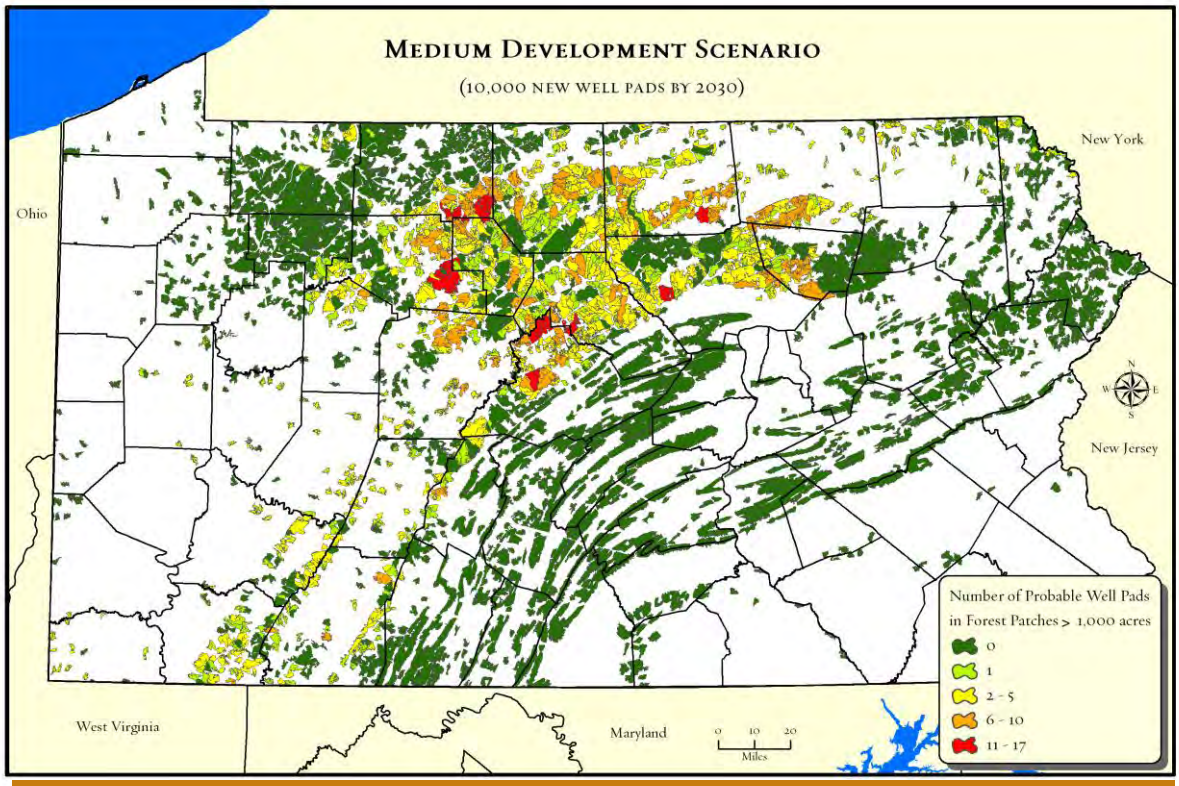
Substantial areas of overlap are indicated between likely future Marcellus development areas and Pennsylvania's most important forest, freshwater, sensitive species habitats, and outdoor recreation sites.

**FORESTS**

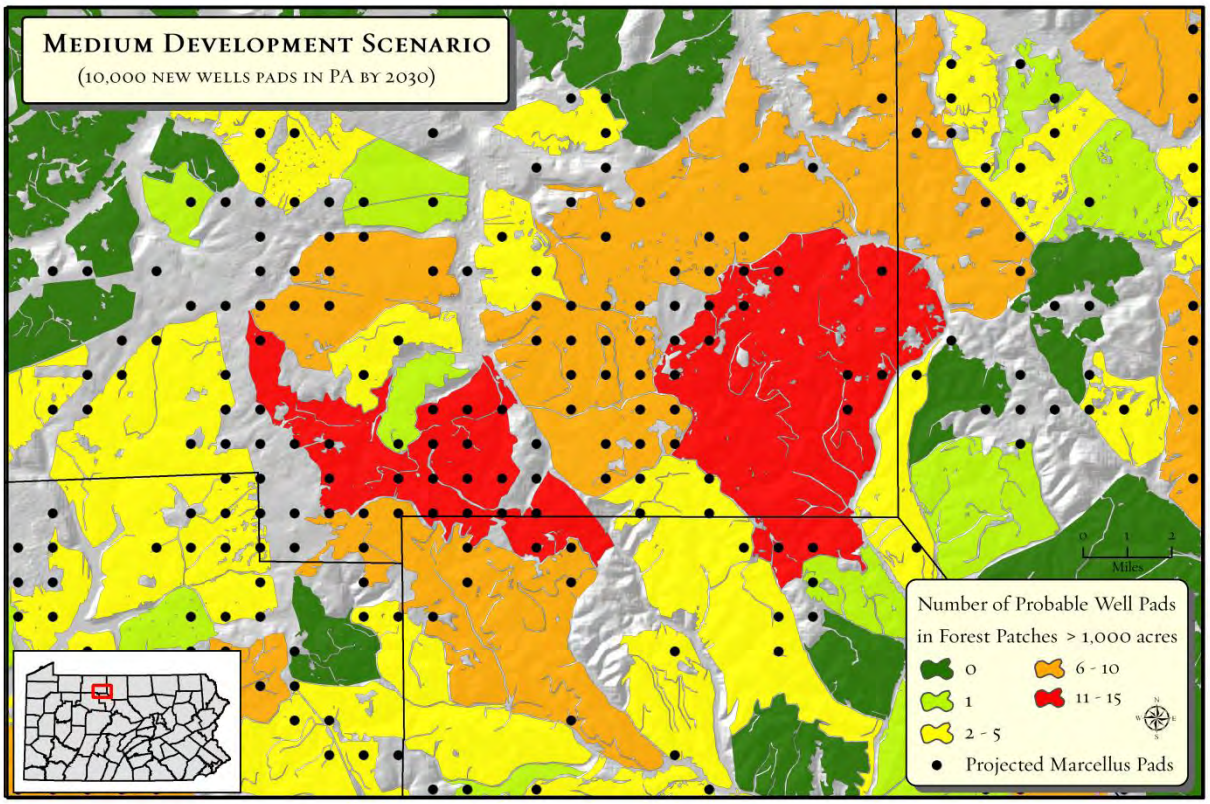
Forests are Pennsylvania's most extensive natural habitat type. Once covering at least 95 percent of the state's land area, forests were whittled away for agriculture, charcoal for iron smelting, and lumber until only a third of the state's forests remained. Forests have rebounded steadily to cover about 60 percent of the state, though a trend toward increasing net loss of forest has emerged during the past decade. Pennsylvania is famous worldwide for its outstanding cherry, oak, and maple hardwoods, and forests provide livelihoods for many thousands of Pennsylvanians in the forest products and tourism industries. They also contribute enormously to the quality of life for all Pennsylvanians by filtering contaminants from water and air, reducing the severity of floods, sequestering carbon dioxide emissions that would otherwise warm the planet, and providing a scenic backdrop to recreational pursuits.

A majority of projected well locations are found in a forest setting for all three scenarios (64% in each case). The low scenario would see 4,310 well pads in forest areas. With an average cleared forest average of 8.8 acres per pad (including roads and other infrastructure), the total forest clearing would be approximately 38,000 acres. Indirect impacts to adjacent forest interior habitats would total an additional 91,000 acres. Forest impacts from the medium scenario (6,950 projected wells in forest locations) would be 61,000 cleared forest acres and an additional 147,000 acres of adjacent forest interior habitat impacts. For the high scenario (10,250 forest well pads), approximately 90,000 acres would be cleared, and an additional 220,000 acres of forest interior habitats would be affected by new adjacent clearings. While the high Marcellus scenario would result in a loss of less than one percent of the state's total forest acreage, areas with intensive Marcellus gas development could see a loss of 2-3 percent of local forest habitats. Some part of the cleared forest area will become reforested after drilling is completed, but there has not been enough time to establish a trend since the Marcellus development started.

Large contiguous forest patches are especially valuable because they sustain wide-ranging forest species, such as northern goshawk, and provide more habitat for forest interior species. They are also more resistant to the spread of invasive species, suffer less tree damage from wind and ice storms, and provide more ecosystem services – from carbon storage to water filtration – than small patches. The Nature Conservancy and the Western Pennsylvania Conservancy's Forest Conservation Analysis mapped nearly 25,000 forest patches in the state greater than 100 acres. Patches at least 1,000 acres in size are about a tenth of the total (2,700) and patches at least 5,000 acres are rare (only 316 patches). In contrast to overall forest loss, projected Marcellus gas development scenarios indicate a more pronounced impact on large forest patches. For example, 40 percent of patches greater than 5,000 acres are projected to have at least one well pad and associated infrastructure located in them in the medium scenario compared to just over 20 percent for patches > 1,000 acres. Most affected large patches have multiple projected well pads (as many as 29). The projections indicate larger patches are likely to be more vulnerable, with over a third projected to have at least one new well pad and road. Many affected large patches have multiple projected well pads (as many as 17 for patches). While one or two well pads and associated infrastructure may not fragment the large patch into smaller patches, each additional well pad increases the likelihood that the large patch will become several smaller patches with a substantially reduced forest interior habitat area.



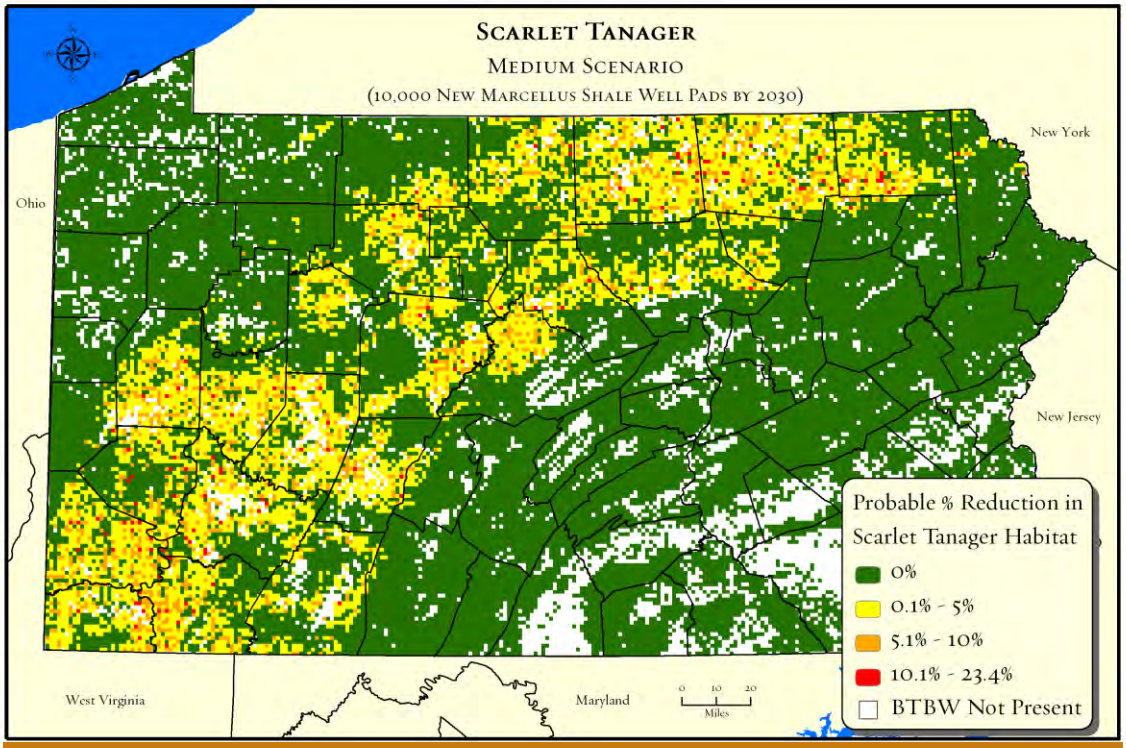

Map showing number of probable Marcellus well pads in forest patches greater than 1,000 acres across Pennsylvania.



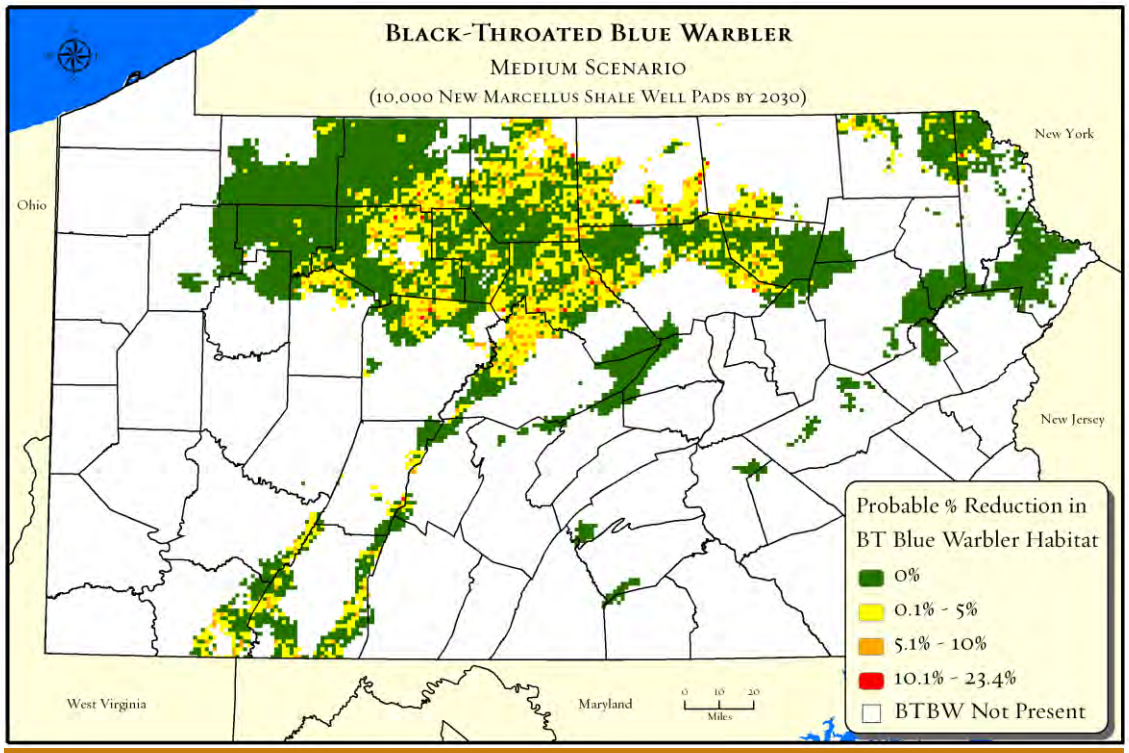

Map showing projected number of well pads in forest patches greater than 1,000 acres under the medium development scenario in Potter, Cameron, McKean and Forest Counties.

Bird species that nest in close canopy forest environments are often referred to as "forest interior" species. The Carnegie Museum of Natural History, Powdermill Nature Reserve and the Pennsylvania Game Commission recently completed Pennsylvania's Second Breeding Bird Atlas project. As part of the project, trained ornithologists conducted point counts using standardized protocols at 39,000 sites from 2004 to 2009. The result is an incredibly detailed data base that provides the most accurate information on the distribution and density of breeding birds available anywhere in the United States. Density data for several forest interior nesting species were mapped and intersected with the projected Marcellus gas well pad locations. The resulting maps show the estimated reduction in habitat for that species in each Marcellus gas probability pixel (including both cleared forest and adjacent edge effects). Scarlet Tanagers are one of the most widespread forest interior nesting bird in the state. Since they are so widespread, a majority of their range in the state is outside of the most likely Marcellus development areas. In some locations, scarlet tanager populations could decline by as much as 23 percent in the Medium Scenario. Black-throated blue warblers are more narrowly distributed in Pennsylvania favoring mature northern hardwood and coniferous forests with a dense understory, frequently in mountain terrain. Since most of their breeding range in Pennsylvania overlaps with likely Marcellus development areas, a higher proportion of their habitat could be affected.

Scarlet tanager © U.S. Fish and Wildlife Service




Map showing estimated percent loss of habitat for Scarlet Tanagers under medium scenario.

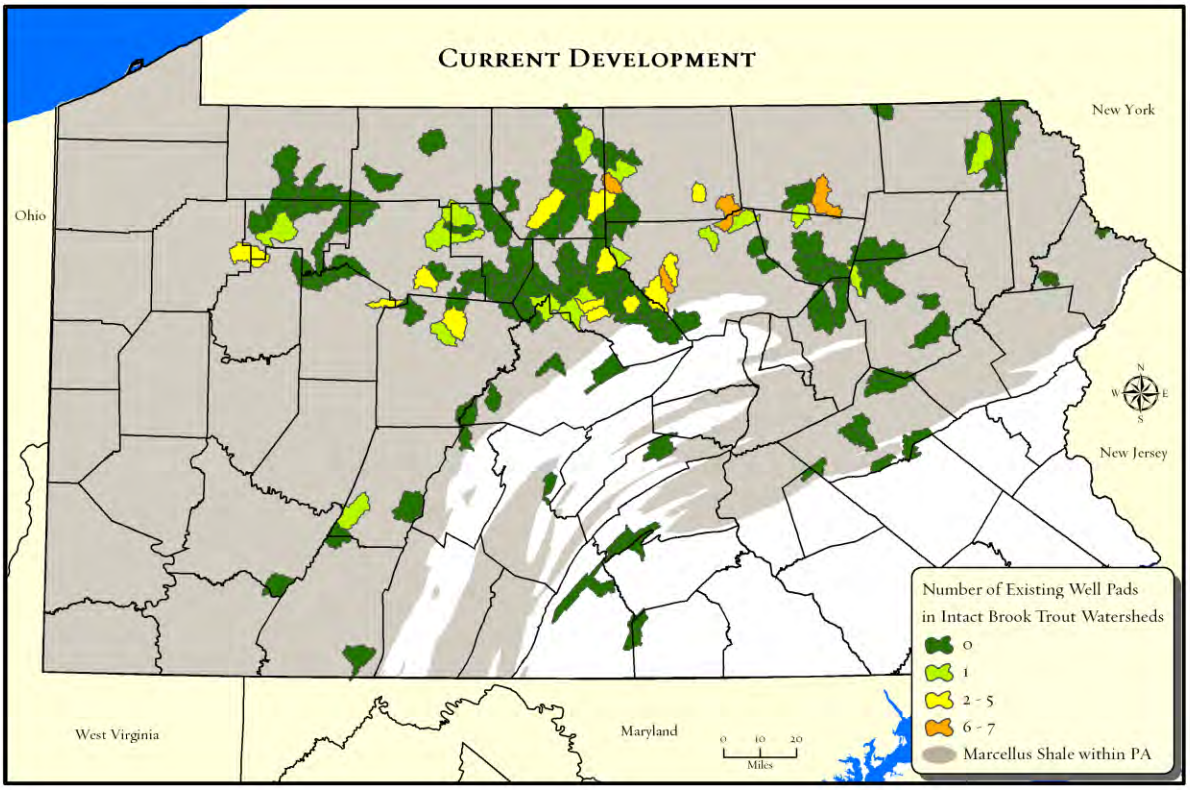



Map showing estimated percent loss of habitat for Black-Throated Blue Warblers under medium scenario.

**FRESHWATER**

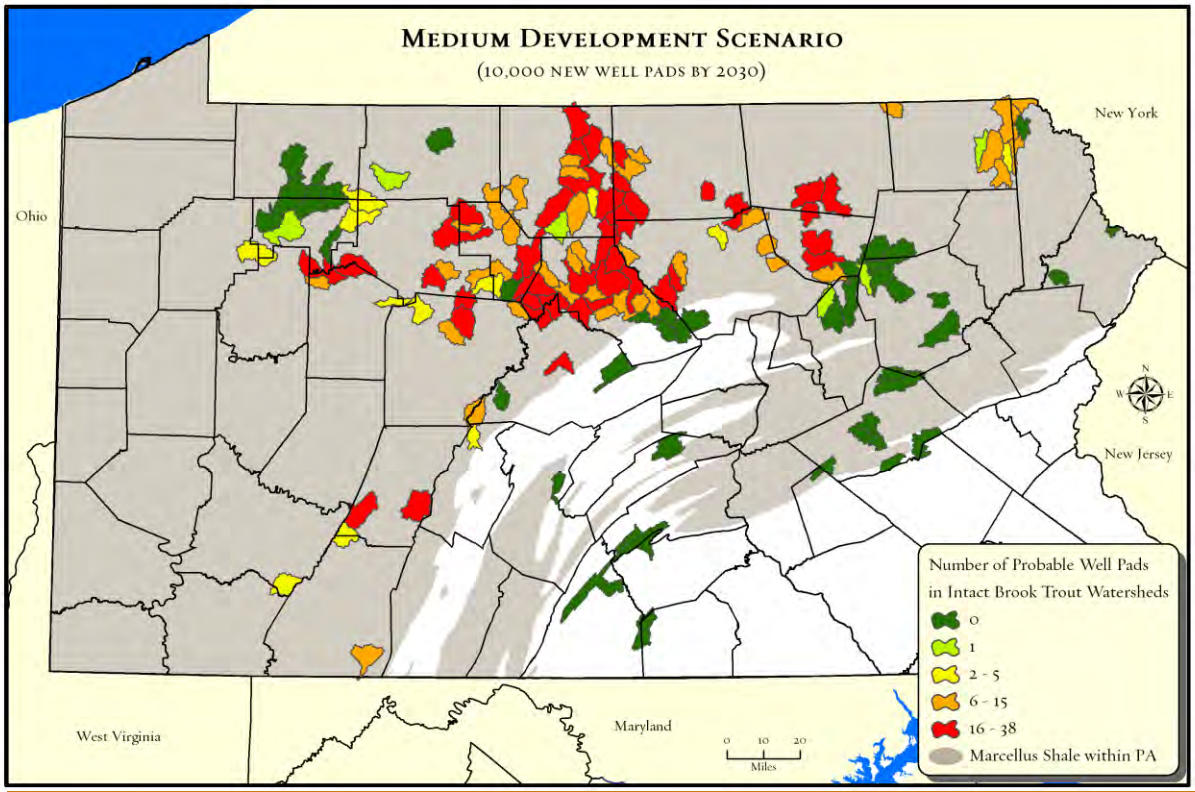
Home to three great river systems and one of the Great Lakes, Pennsylvania's fresh water resources are vital not only to the Commonwealth but to much of the eastern United States. The ***Ohio River*** basin contains the richest fresh water ecosystems in North America. In Pennsylvania, French Creek and parts of the Upper Allegheny River contain some of the most intact aquatic ecosystems in the entire basin. The ***Susquehanna* River** is the source of more than half the fresh water that enters the Chesapeake Bay, and most of the water that flows down the Susquehanna River originates in tributary headwaters across a wide swath of central Pennsylvania. Forming Pennsylvania's eastern boundary, the ***Delaware River*** is the longest undammed river in the eastern United States, one of the last strongholds for Atlantic coast migratory fish, and provides the drinking water source for nearly 20 million Americans living in Pennsylvania, New York, and New Jersey. Because of their importance to human health and livelihoods, the potential of Marcellus gas development to affect water flows and quality have received growing attention from regulatory agencies, natural gas companies, and environmental groups.

The intersection of gas development with sensitive watersheds has received less attention. High Quality and Exceptional Value (EV) watersheds have been designated by the Pennsylvania Department of Environmental




Map showing current number of Marcellus well pads in intact and predicted intact brook trout watersheds. Data source: Eastern Brook Trout Joint Venture.

Protection across the state. Our projections indicate 28 percent of High Quality and 5 percent of Exceptional Values streams have or will have Marcellus gas development during the next two decades. Presence of well pads in these watersheds may not be a problem as long as spill containment measures and erosion and sedimentation regulations are strictly observed and enforced in these areas. More specifically, the projections indicate 3,581 well pads could be located within ½ mile of a High Quality or Exceptional Values streams. Pads within close proximity to High Quality and especially Exceptional Value streams pose more risk than those at greater distances, as there is increased risk for potential spills and uncontained sediments to find their way into streams.




Map showing projected number of Marcellus well pads by 2030 in intact and predicted intact brook trout watersheds under medium scenario. Data source: Eastern Brook Trout Joint Venture.

Native brook trout are one of the most sensitive aquatic species in Pennsylvania watersheds. Brook trout favor cold, highly-oxygenated water and are unusually sensitive to warmer temperatures, sediments, and contaminants. Once widely distributed across Pennsylvania, healthy populations have retreated to a shrinking number of small watersheds. Many of these watersheds overlap with the Marcellus shale formation. A large majority (113) of the 138 intact or predicted intact native brook trout watersheds in Pennsylvania are projected to see at least some Marcellus gas development. Over half (74) are projected to host between 6 – 38 well pads, and the number reaches as high as 64 pads for some intact brook trout watersheds in the high scenario. Rigorous sediment controls and carefully designed stream crossings will be critical for brook trout survival in watersheds, especially upper watersheds, with intensive Marcellus development.

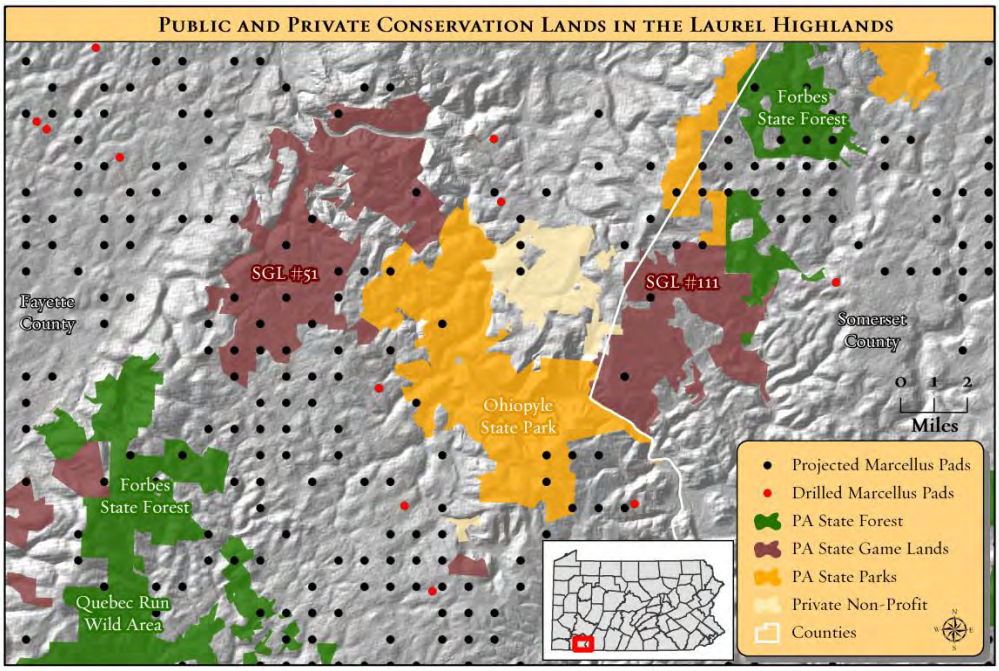
**RARE SPECIES**

Green salamander © Pennsylvania Fish and Boat Commission

Of the approximately 100,000 species believed to occur in Pennsylvania, just over 1 percent (1052) is tracked by The Pennsylvania Natural Heritage Program (PNHP). Due to low population sizes and immediate threats, these species are rare, declining or otherwise considered to be of conservation concern. PNHP records indicate that 329 tracked species have populations within pixels that have a relatively high modeled probability for Marcellus development. Nearly 40 percent (132) are considered to be globally rare or critically endangered or imperiled in Pennsylvania. Many are found in riparian areas, streams, and wetlands, while others are clustered in unusually biologically diverse areas such as the Youghiogheny Gorge. Some of these species may have only one, two or three populations left in the state. Two examples include the green salamander (*Aniedes aeneus)* with all known populations in relatively high probability Marcellus development pixels and snow trillium (*Trillium nivale*) with 73 percent of known populations in relatively high probability pixels. A well-managed screening system to identify the presence of these species and their preferred habitats will be critical to their survival as energy development expands across the state.

**RECREATION**

Pennsylvania has built one of the largest networks of public recreation lands in the eastern United States, but much of it could see Marcellus and other natural gas development in coming decades. Of the 4.5 million acres of state and federal lands in the state, we estimate as little as 500,000 acres are permanently protected from surface mineral development, including gas drilling. State and federal agencies do not own mineral rights under at least 2.2 million acres. Most other areas where the state does own mineral rights can be leased, such as the estimated 700,000 acres previously leased for gas development on state forest lands. Severe budget pressures will likely to tempt the legislature to lease additional lands in the future. Our projections excluded state Wild and Natural Areas, National Park lands, and Congressionally-designated Wilderness Areas but otherwise assumed that high probability Marcellus gas pixels on public lands could be developed. The low scenario projects 897 pad locations on State Forest and State Game Lands which expands to 1,438 well pads in the medium scenario and 2,096 pads in the high scenario. The focal area below illustrates what the overlap of future gas development and conservation lands could look like in the medium scenario for the southern Laurel Highlands. It projects 7 well pads in the portion of Forbes State Forest visible in the focal area above, 13 pads on State Game Lands 51, and 3 on State Game Lands 111.




Map showing projected Marcellus well pads under the medium scenario on public and private conservation lands in the Laurel Highlands.

Pennsylvania's state park system, recognized as one of the best in the nation, illustrates the challenge of protecting recreational values in areas of intensive Marcellus development. While the DCNR has a long standing policy of not extracting natural resources in state parks, it does not own the mineral rights under an estimated 80 percent of the system's 283,000 acres. Our projections indicate Marcellus well pads could be located in between 9 and 22 state parks.

**AVOIDING FOREST IMPACTS IN THE LAUREL HIGHLANDS**

The projected potential impacts of Marcellus gas energy development assume recent patterns of development will continue. Given the relatively large areas drained by Marcellus gas pads (depending on the lateral length and number of wells per pad), there is flexibility in how they are placed. This allows us potentially to optimize between energy production and conservation outcomes. To look at how conservation impacts could be minimized, we examined how projected Marcellus gas pads could be relocated to

| Projected Well Pads on State Lands (Medium Scenario) | |
|---|---|
| **DCNR State Forests** | **1,002** |
| **DCNR State Parks** | **41** |
| **State Game Lands** | **436** |
| **Total State Lands** | **1,479** |

avoid forest patches in the Southern Laurel Highlands in Fayette and Somerset counties. This area is important because it represents a unique ecological region with a large amount of state land as well as private farmland and forest land. The area is also facing great pressure to develop the Marcellus Gas resource. The focus area included approximately 350 square miles and included Chestnut Ridge on its western border and Laurel Ridge on its east. Within the area, there are two state parks (Ohiopyle State Park and Laurel Hill State Park), two State Game Lands (SGL 51, SGL 111), and state forest land (Forbes State Forest).

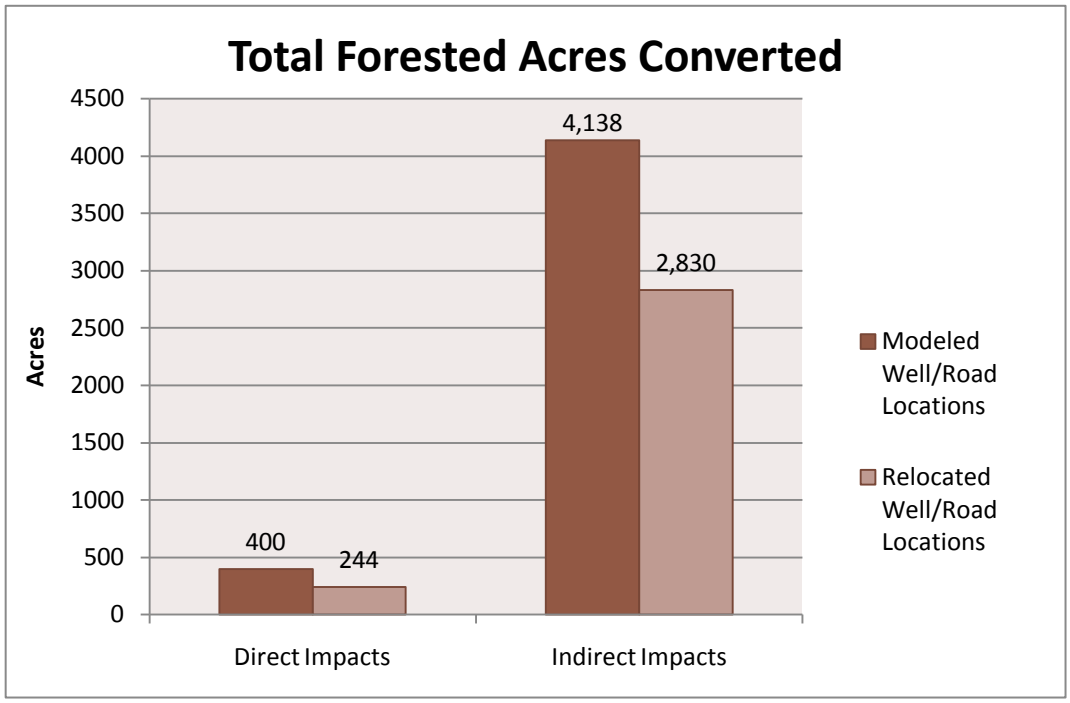
The Medium Scenario projected 127 well pads in the focus area. Fourteen well pads were projected in agricultural fields, 33 were in edge habitat (within 100 m of the forest edge), 11 fell within existing cleared areas (e.g. strip mines), and 69 were in forest. There were five pads on Ohiopyle State Park, and 13 within a mile of its boundary. Laurel Ridge State Park contained two pads. Forbes State Forest had seven modeled pads. State Game Lands 111 had 3 pads, and SGL 51 had 13. It was not clear if DCNR State Parks Bureau or the Game Commission control the sub-surface mineral rights beneath the 23 modeled pads. Given that 80 percent of mineral rights are severed on State Park and State Game Lands (and close to 100 percent in western parts of the state), we have assumed that drilling could happen at those projected locations.

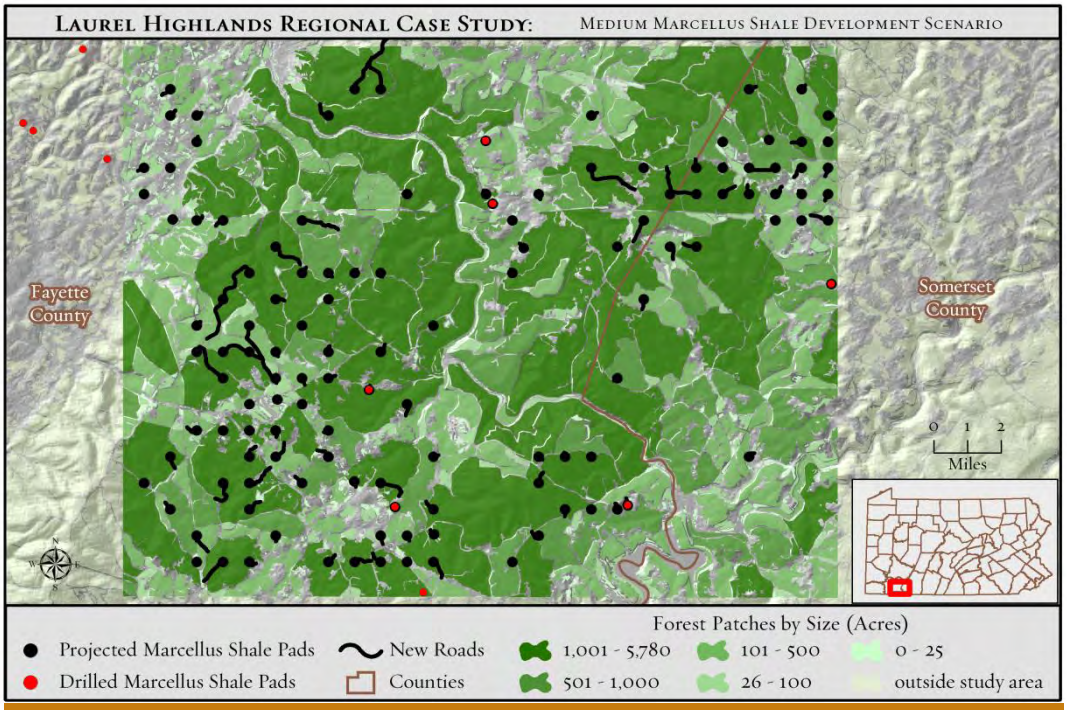
To assess additional impacts beyond the well pad itself, we placed a new and/or improved road from the projected pad to the nearest existing road (ESRI Roads Layer). We placed new roads along existing trails, paths and openings whenever detectable on aerial photo imagery (used Bing Maps and 2005-2006 PA Map imagery), avoiding wetlands, steep slopes, cliffs, rock outcrops, and buildings, and where possible, rivers, streams, and forest patches. The projected pads and roads required clearing 400 acres of forest.

Can a modest shift in the location of well pads reduce impacts to forest patches and conservation lands? To reduce the impacts to forest habitats, the wells were relocated to nearby existing anthropogenic openings, old fields, or agricultural fields. Attempts were made to maintain the 4,200 foot (1,260 m) distance between modeled wells. If nearby open areas did not exist, the locations of the well pads were moved toward the edges of forest patches to minimize impacts to forest interior habitats. A set of rules was developed and followed to minimize bias, including:

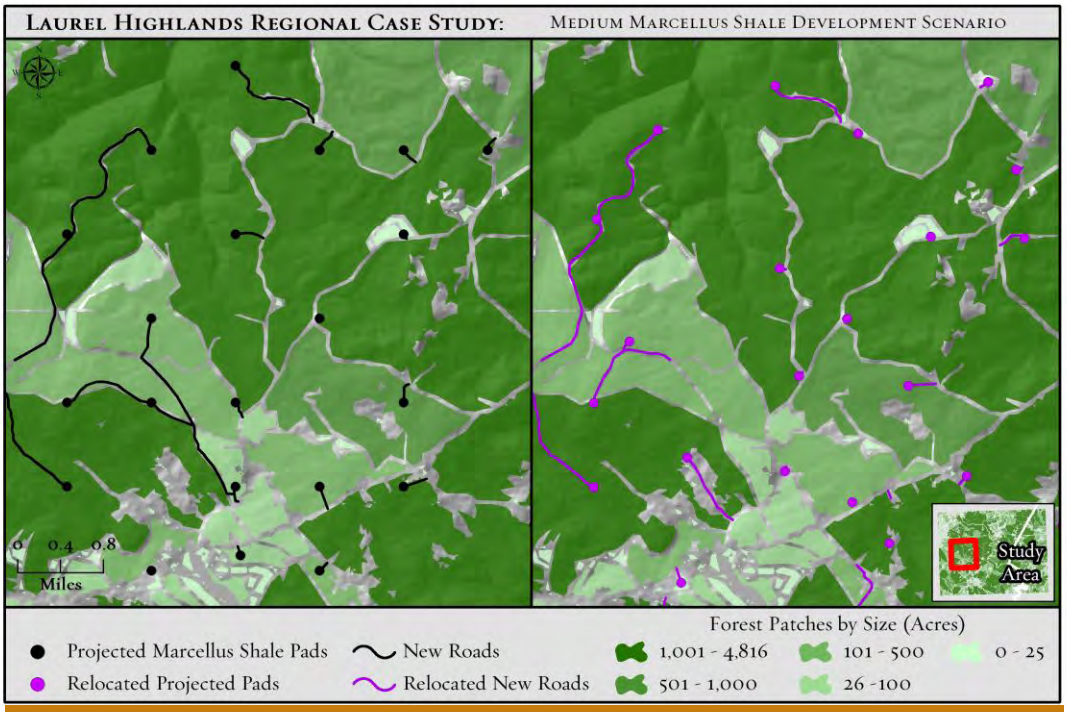
1. Modeled well pads were not relocated if they occurred in old fields or agricultural fields.
2. Modeled well pads that occurred in forest or edge habitat were moved but well pads were placed in the same general areas as the modeled well pad;
3. Attempts were made to avoid placing relocated well pads any closer that the minimum distance between pads, as specified by the medium scenario (1260 m )
4. Agriculture, cleared land (e.g., former strip mines), or otherwise opened land cover was favored over forest or edges for relocating well pads;
5. If the well pad could not be placed in an open area, forest edges were favored over deep interior forest;
6. Residential areas were avoided. Relocated well pads were placed at least 500 feet (150 m) from homes;
7. Wetlands, water, steep slopes, cliffs, rock outcrops, creeks and rivers, buildings and manicured lawns were avoided;
8. Relocated well pads were only placed in areas with similar to those that supported modeled pads.
9. Relocated well pads often were connected to roads using existing trails, paths and openings whenever detectable on aerial photo imagery (used Bing Maps and 2005-2006 PA Map imagery);
10. The same number of relocated well pads were placed on state lands and Western Pennsylvania Conservancy lands as they were in the modeled output;
11. When the modeled well pad occurred within a forest patch with no nearby alternative locations (due to proximity of other wells or environmental constraints), the projected well pad was not relocated.

The relocated wells and roads did not eliminate forest impacts in this heavily forested landscape, but there was a significant reduction. Total forest loss declined almost 40% while impacts to interior forest habitats adjacent to new clearings declined by a third.






Location of 127 projected Marcellus well pads and new roads in the study area in the southern Laurel Highlands.




Relocated well pads (on the right) reduced forest clearing and forest interior habitat impacts by 40 % and 33% respectively compared to the projected well pads (on the left).

## Key Findings

Key findings from the Pennsylvania Energy Impacts Assessment for Marcellus Shale natural gas include:

- About 60,000 new Marcellus wells are projected by 2030 in Pennsylvania with a range of 6,000 to 15,000 well pads, depending on the number of wells per pad;

- Wells are likely to be developed in at least 30 counties, with the greatest number concentrated in 15 southwestern, north central, and northeastern counties;

- Nearly two thirds of well pads are projected to be in forest areas, with forest clearing projected to range between 38,000 and 90,000 acres depending on the number of number of well pads that are developed. An additional range of 91,000 to 220,000 acres of forest interior habitat impacts are projected due to new forest edges created by well pads and associated infrastructure (roads, water impoundments);

- On a statewide basis, the projected forest clearing from well pad development would affect less than one percent of the state's forests, but forest clearing and fragmentation could be much more pronounced in areas with intensive Marcellus development;

- Approximately one third of Pennsylvania's largest forest patches (>5,000 acres) are projected to have a range of between 1 and 17 well pads in the medium scenario;

- Impacts on forest interior breeding bird habitats vary with the range and population densities of the species. The widely-distributed scarlet tanager would see relatively modest impacts to its statewide population while black-throated blue warblers, with a Pennsylvania range that largely overlaps with Marcellus development area, could see more significant population impacts;

- Watersheds with healthy eastern brook trout populations substantially overlap with projected Marcellus development sites. The state's watersheds ranked as "intact" by the Eastern Brook Trout Joint Venture are concentrated in north central Pennsylvania, where most of these small watersheds are projected to have between two and three dozen well pads;

- Nearly a third of the species tracked by the Pennsylvania Natural Heritage Program are found in areas projected to have a high probability of Marcellus well development, with 132 considered to be globally rare or critically endangered or imperiled in Pennsylvania. Several of these species have all or most of their known populations in Pennsylvania in high probability Marcellus gas development areas.

- Marcellus gas development is projected to be extensive across Pennsylvania's 4.5 million acres of public lands, including State Parks, State Forests, and State Game Lands. Just over 10 percent of these lands are legally protected from surface development.

- Integration of conservation features into the planning and development of Marcellus gas well fields can significantly reduce impacts. For example, relocating projected wells to open areas or toward the edge of large forest patches in high probability gas development pixels in the southern Laurel Highlands reduces forest clearing by 40 percent and forest interior impacts by over a third.

## Additional Information

- Geologic information on the Marcellus shale formation in Pennsylvania: http://www.dcnr.state.pa.us/topogeo/oilandgas/marcellus_shale.aspx

- Estimates of Marcellus shale formation gas reserves: http://geology.com/articles/marcellus-shale.shtml

- Baker-Hughes weekly oil and gas rig count http://gis.bakerhughesdirect.com/Reports/StandardReport.aspx

- Pennsylvania Department of Environmental Protection, Permit and Rig Activity Report: http://www.dep.state.pa.us/dep/deputate/minres/oilgas/RIG10.htm

- Copeland, H. E., K.E. Doherty, D.E. Naugle, A. Pocewicz, and J. M. Kiesecker. 2009. Mapping Oil and Gas Development Potential in the US Intermountain West and Estimating Impacts to Species: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0007400

- Overview of forest fragmentation impacts on forest interior nesting species: http://www.state.nj.us/dep/fgw/neomigr.htm

- Overview of Pennsylvania High Quality and Exceptional Value Streams: http://www.dcnr.state.pa.us/wlhabitat/aquatic/streamdist.aspx

- Pennsylvania Department of Environmental Protection, Chapter 93 Water Quality Standards, Exceptional Value and High Quality Streams: data downloaded from Pennsylvania Spatial Data Access: http://www.pasda.psu.edu

- Eastern Brook Trout Joint Venture intact brook trout watersheds: http://128.118.47.58/EBTJV/ebtjv2.html

- Overview of Carnegie Museum of Natural History, Powdermill Nature Reserve, and the Pennsylvania Game Commission's 2nd Pennsylvania Breeding Bird Atlas Project: http://www.carnegiemnh.org/powdermill/atlas/2pbba.html

- Pennsylvania Natural Heritage Program, including lists of globally rare and state endangered and imperiled species: http://www.naturalheritage.state.pa.us/

- U.S. Department of Agriculture, Natural Resources Conservation Service, National Agriculture Imagery Program: http://datagateway.nrcs.usda.gov/GDGOrder.aspx

- DigitalGlobe, GlobeXplorer, ImageConnect Version 3.1: http://www.digitalglobe.com

# Wind

Wind has become one of the country's fastest growing sources of renewable energy. Pennsylvania is a leader in the industry as host to several wind company manufacturing plants and corporate headquarters. Wind energy development has been spurred by its potential to reduce carbon emissions, promote new manufacturing jobs, and increase energy independence. Technological advances have expanded the size and efficiency of wind turbines during the past decade. This, together with state and federal incentive programs, has facilitated wind development in Pennsylvania, which otherwise ranks relatively low among states for its potential wind generation capacity. The eight turbines installed next to the Pennsylvania Turnpike in Somerset County a decade ago have grown to nearly 500 turbines, with more permitted for construction (AWEA, 2010). Topography is a key factor in average wind speeds across Pennsylvania, so nearly all turbines have been built on mountain ridgelines or on top of high elevation plateaus.

Wind energy has become the most symbolic icon of the shift toward a low carbon economy. With no air emissions or water consumption, it is one of the cleanest renewable energy types. Communities across the state benefit economically as rural landowners lease their properties, skilled jobs are created to manufacture turbines, and workers are hired to install and maintain turbines. Wind development has faced controversy in some areas from neighboring landowners and those worried about impacts to migrating birds and bats. The wind industry, government agencies, and independent researchers have invested considerable effort in trying to better understand impacts on birds and bats. For example, 26 wind development companies have signed a cooperative agreement with the Pennsylvania Game Commission to conduct bird, bat and animal surveys using specified protocols in proposed development areas. Among other findings have been the discovery of the Pennsylvania's second largest Indiana bat maternal colony and a variety of previously undocumented foraging and roosting locations for the state's two rarest bats (Indiana and eastern small-footed). Less understood are the potential habitat impacts of wind development in the northeastern United States. This assessment, therefore, focuses on impacts to forest and stream habitats and selected species of conservation concern that may be vulnerable to development of ridgetop habitats.

## What is Wind Energy?

Wind mills have powered grain processing and water pumping in agriculture around the world – most famously in the Netherlands – for centuries. The first modern wind facilities to generate electricity were built in California in the early 1980s. Rated at less than 0.5 MW capacity per turbine, the towers were only 50 feet tall. These facilities were poorly designed and generated considerable controversy because they caused significant mortalities to migrating hawks and eagles. Wind energy development did not expand appreciably until the late 1990s when newer turbine designs and federal energy incentives stimulated the development of new facilities. These turbines were rated at 1.0 or 1.5 MW capacity and reached about 200 feet high at the tip of their rotor. Since the power produced by a wind turbine is proportional to the cube of the blade size and how high in the air it is; turbine size, height and power ratings have expanded steadily. The largest turbines installed in Pennsylvania are now rated at

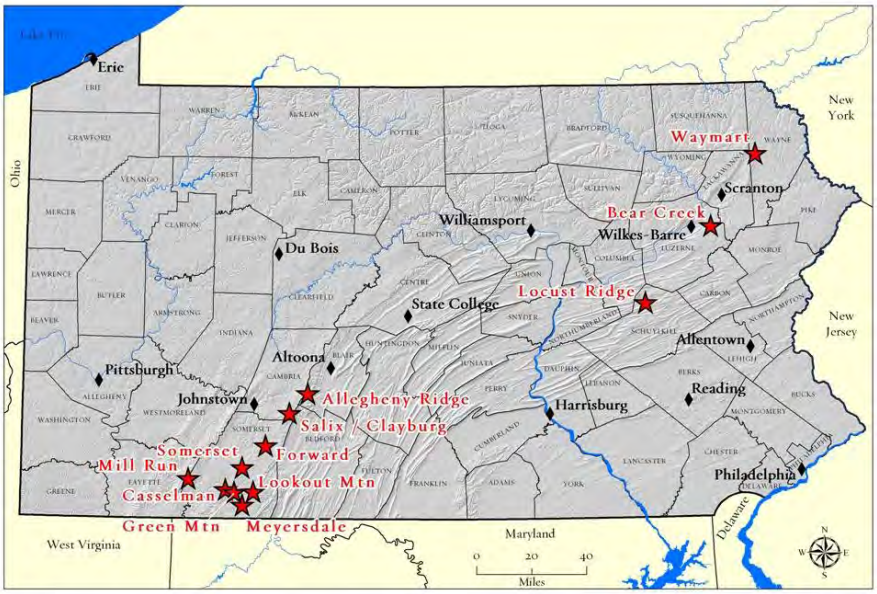
2.5 MW (the average was 1.8 MW in 2009) and reach over 400 feet to the tip of the rotor at the apex of its rotation.

Location is everything for wind development in the northeastern United States. Unlike the vast windswept plains in the Midwest and the intermountain West, high wind speeds in the Northeast are primarily confined to mountain ridgetops, plateau escarpments, and the Atlantic and Great Lake shorelines. Areas that have a wind power class rating of 3 or more (300 watts per $m^2$) are potentially feasible for wind power development. Wind companies will lease areas that seem to have the most favorable characteristics including wind class, flat pad sites, proximity to transmission lines, and proximity to existing highways. Before development, a wind development company will typically place an anemometer tower on potential development sites to improve knowledge about wind power at the site during a year or longer monitoring period. The turbines are mounted on pads at least 800 feet apart with an access road between towers. The average size of wind facilities has been growing steadily since the first eight were established in 2000. The two largest facilities are now between 75 and 100 turbines.

Several steps have been taken to address potential conflicts between wind development and wildlife in Pennsylvania. The Pennsylvania Game Commission (PGC) has a voluntary agreement in place with most wind companies active in the state to screen proposed facilities for possible impacts to birds and bats and migratory pathways. Participating wind companies carry out pre-construction monitoring for birds and bats. If possible conflicts are identified, PGC works with wind companies to avoid or minimize impacts and to continue monitoring post construction in some cases. Second, the Pennsylvania Wind and Wildlife Collaborative (PWWC) was established in 2005 with a state goal to develop a set of "Pennsylvania-specific principles, policies and best management practices, guidelines and tools to assess risks to habitat and wildlife, and to mitigate for the impact of that development." Several studies on wildlife and habitat issues have been commissioned, though guidelines and Best Management Practices (BMPs) have not been released.

## Current and Projected Wind Energy Development

We documented the spatial foot print of 319 wind turbines at 12 wind facilities across the state by comparing aerial photos taken before and after development. Turbine pads, roads, and other new clearings were digitized for all 12 facilities visible in 2008 images from the

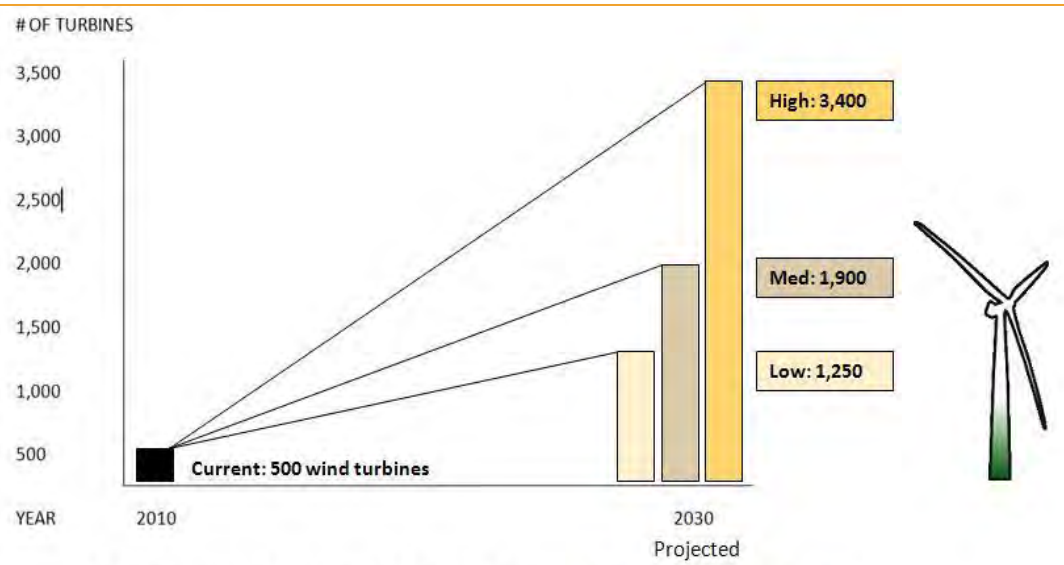



Map showing 12 wind facilities included in the spatial footprint analysis.

| Average Spatial Disturbance for Wind Energy Development in Forested Context (acres) | | |
|---|---|---|
| Forest cleared for wind turbine | 1.4 | 1.9 |
| Forest cleared for associated infrastructure (roads, other cleared areas) | 0.5 | |
| Indirect forest impact from new edges | 13.4 | |
| TOTAL DIRECT AND INDIRECT IMPACTS | 15.3 | |

National Agriculture Imagery Program. The ground excavated for turbines, roads, and associated infrastructure (e.g., clearings for constructions staging areas or electrical sub-stations) is the most obvious spatial impact. For each turbine site, turbine pads, new roads, staging areas, and sub-stations were digitized and measured. Turbine pads occupy 1.4 acres on average, while the associated infrastructure (roads, staging areas, and substations) takes up an additional half acre, for a total of 1.9 acres of spatial impact per wind turbine.

As with Marcellus gas development, adjacent lands can also be impacted even if they are not directly cleared (See p. 11 for a description of forest edge impacts on forest "interior" species). To assess the potential interior forest habitat impact, we created a 330 foot buffer into forest patches from new edges created by wind turbine and associated infrastructure development. For turbine sites developed in forest areas (about 80% of the 319 turbines), an average area of 13.4 acres of interior forest habitat was lost in addition to the 1.9 acres of directly cleared forest.




Key drivers: Alternative Energy Portfolio Standard (AEPS) and projected contribution of wind to meeting that standard.

We project between 1,250 and 3,400 total wind turbines will be erected in Pennsylvania by 2030.

The number of wind turbines built in Pennsylvania will certainly expand during the next two decades. Various factors will drive exactly how many turbines are ultimately built including electricity prices, state and federal incentives, technological improvements, energy and climate policy, regulatory changes, and social preferences. Our projections assume economic, policy, and social conditions will remain favorable enough to promote steady expansion of wind development in the state since we cannot reasonably forecast energy prices, technological developments, and policy conditions. The key driver in our low scenario is that companies will use wind energy to meet 70 percent of the current Alternative Energy Portfolio Standard (AEPS) Tier 1 standard (8 percent of electric generation). This projection indicates an additional 750 turbines (2 MW average) will be added to the 500 turbines currently operating. The key driver in our medium scenario is that utilities will use wind energy to meet 70 percent of an expanded AEPS 15% Tier 1 standard, as proposed in recent draft legislation. That scenario would add 1,400 new turbines to those already built. The high scenario used in this assessment is based on the 20% wind power electric generation scenario used by the National Renewable Energy Laboratory in the Eastern Wind Integration Study (EWITS). This scenario would require 2,900 additional turbines.

Where are those new turbines in each scenario more and less likely to go? To start, we created a probability surface by looking at a range of variables that might be relevant to a company's decision to develop a wind facility with wind turbines that have already been built. We used the maximum entropy modeling approach used to develop the Marcellus gas probability surface (see p. 13) and built the model using 580 existing and permitted wind turbines. Variables that potentially drive wind energy development were chosen based on data availability and included wind power ($W/m^2$), distance to transmission lines, percent slope, distance to roads, and land cover. An additional 193 existing and permitted wind turbines were used to test the validity of the model's probability surface and the model was found to be 95.8% accurate in predicting existing and permitted turbines from randomly sampled undeveloped areas. The resulting probability map indicates many long, narrow high probability sites along ridge tops, and several wider areas on high plateaus and along the Lake Erie coastline.

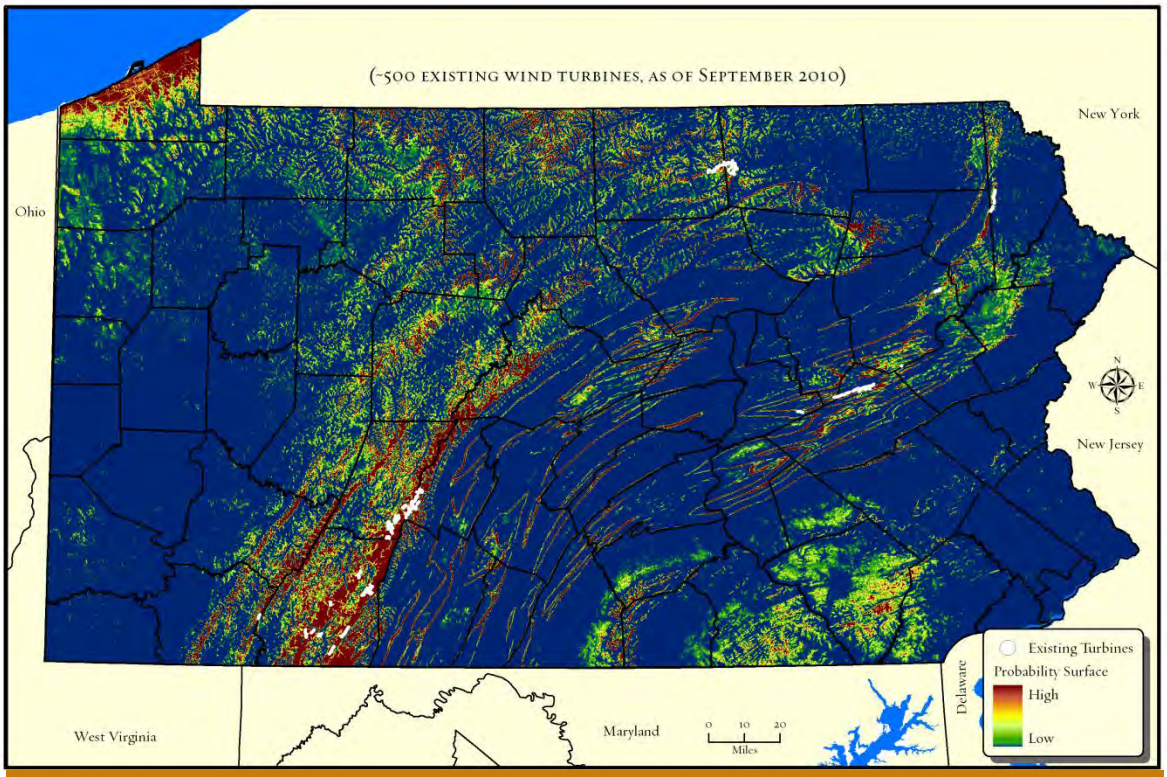
To determine where wind development is more likely, we searched for the highest probability areas where wind turbines in each scenario might be located. The probability surface was re-sampled from 30-meter to 60-meter resolution (0.89 acres) to represent the approximate geographic footprint of wind turbines based on aerial photo assessment. We selected the highest probability pixel, buffered that pixel by a minimum separation distance of 800 feet (240 meters – the average minimum distance between existing turbines), and then selected the next highest probability pixel, and so on. Pixels were selected until the threshold for each scenario was reached (low – 700 turbines; medium – 1,200 turbines; high – 2,700 turbines). The selected pixels were then converted into points for map display purposes.

The resulting projected turbine locations occurred in strings, groups, or scattered single turbines, mostly in southwest, north central and northeastern parts of Pennsylvania. Wind turbines, however, are almost always located in clusters rather than widely separated locations for individual turbines. In order to represent viable wind farms, we selected clusters of pixels with high probability to represent viable wind facilities, based on the following:

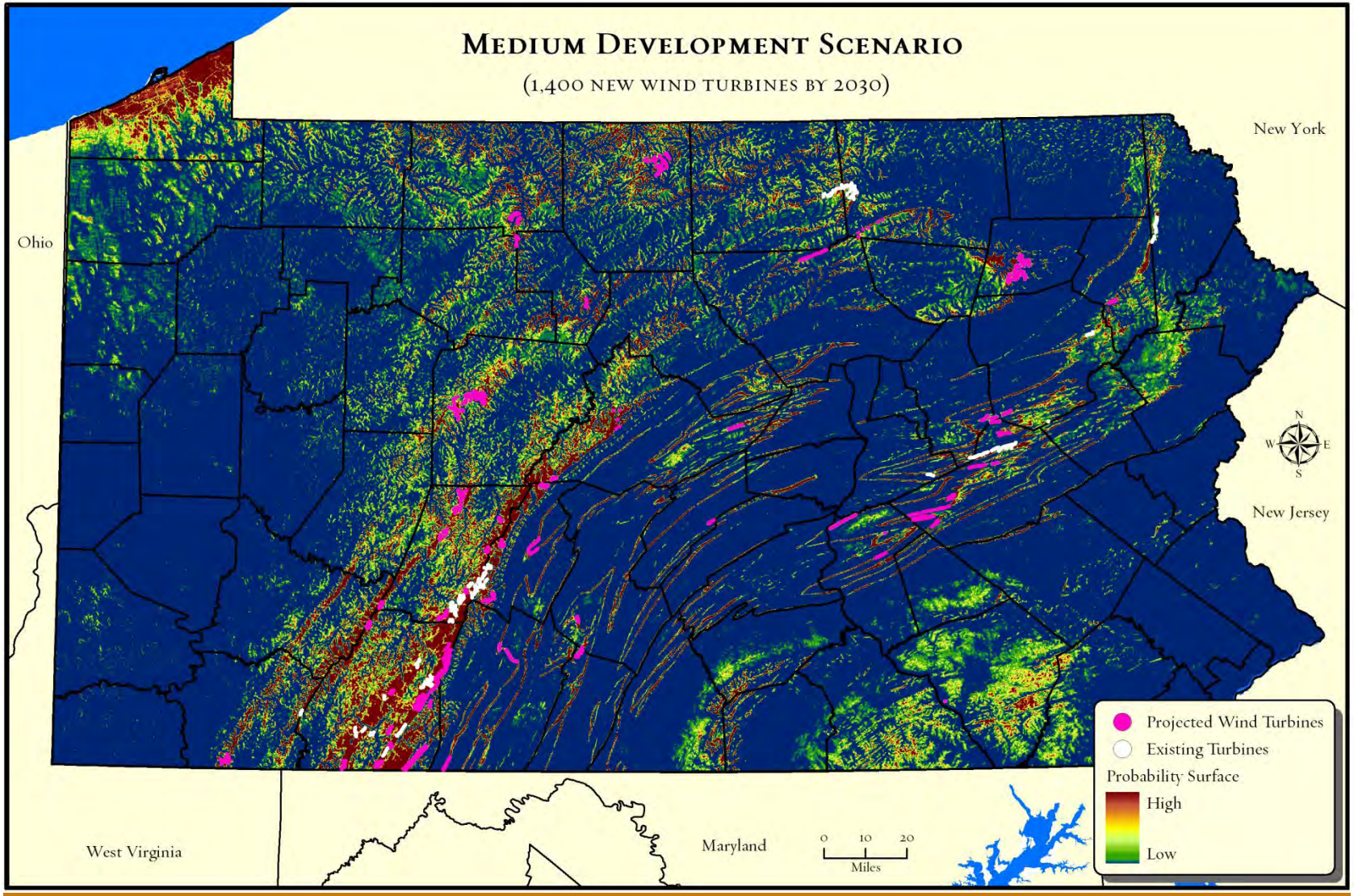
- Excluded areas approximately 300 meters (1,000 ft) from existing homes (as visible in aerial imagery)
- Excluded buffers of regional airports by 6,096 m (20,000 ft) and local airports by 3,048 m (10,000 ft)
- Excluded buffers of existing turbines (buffer = 960 m or 4 times the minimum turbine separation distance of 240 m)
- Excluded Pennsylvania State Parks, Pennsylvania State Forests, and Pennsylvania State Game Lands
- Excluded setbacks of 152 m (500 ft) from the boundaries of state and federal lands

- Required a minimum of 6 projected turbines grouped together to be considered a potentially viable site
- Selected already proposed wind turbines (based on permit data from the Federal Aviation Administration)

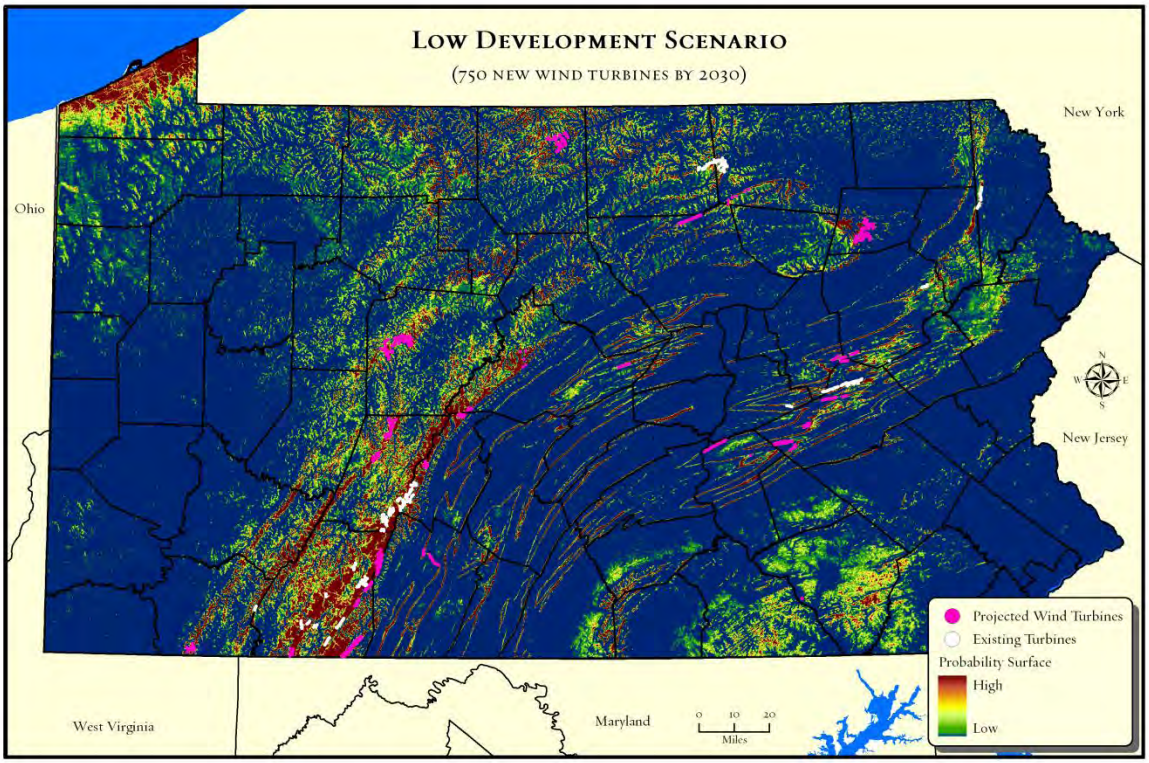
Potential wind facilities were manually selected by identifying groupings of projected wind turbines. Scenarios are cumulative, so the medium scenario includes turbines in both the low and medium scenarios, whereas the high scenario includes all projected turbines.



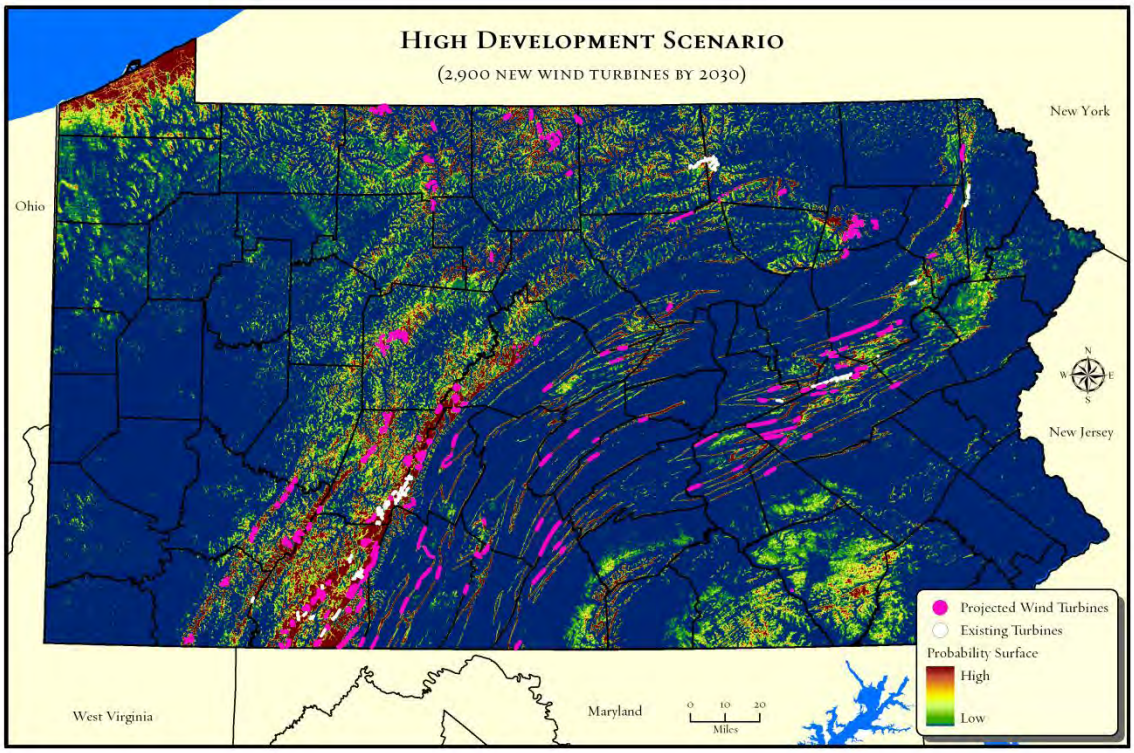

Map showing existing wind turbines with the probability that a given area will be developed indicated by color (dark red is high probability; dark blue is low).



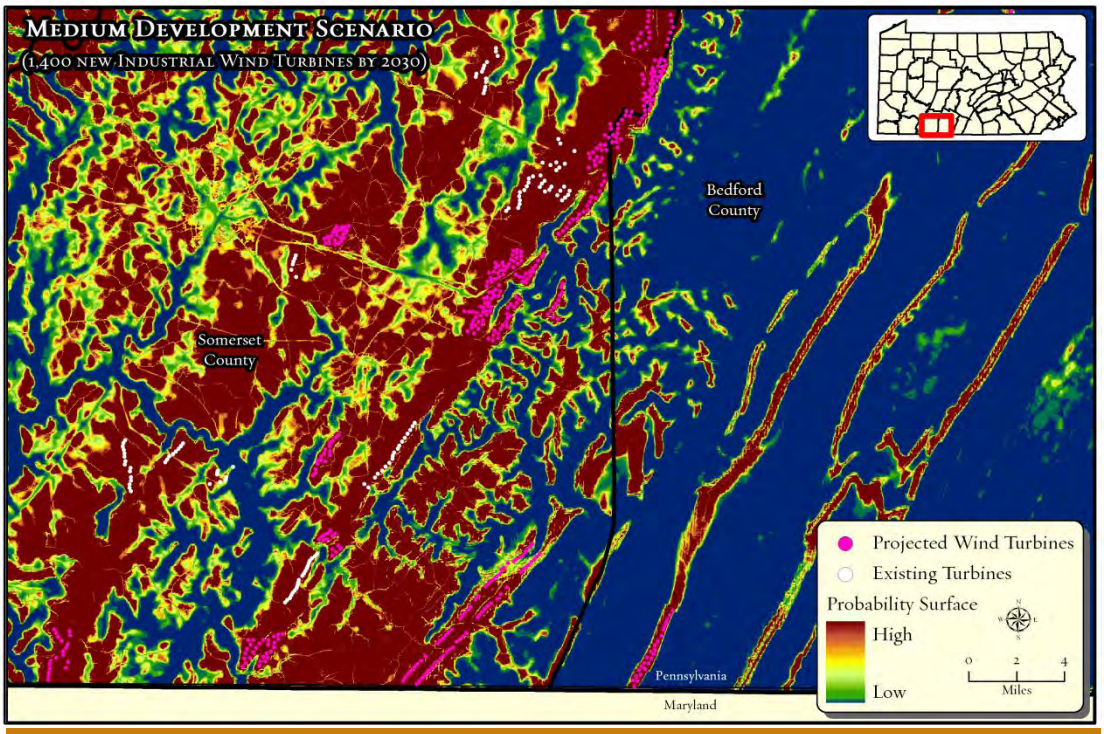

Map showing 1,400 new wind turbines projected by 2030 under the medium development scenario.




Map showing 750 new wind turbines projected by 2030 under the low development scenario.




Map showing 2,900 new wind turbines projected by 2030 under the high development scenario.




Map showing medium wind development scenario within Somerset and Bradford counties.

These geographic projections of future wind energy development are spatial representations of possible scenarios. They are not predictions. We faced several constraints in developing the geographic scenarios:

- We do not have the detailed wind power data that wind companies have developed through anemometer tower monitoring.
- We do not have the detailed location of wind energy leases.

Still, we believe the overall geographic patterns in the projected wind development locations are relatively robust for several reasons. We used over 500 existing or permitted wind turbines to build the model and nearly 200 additional existing and permitted wind turbine sites were used to validate the model. This is typically a sufficient sample size for building predictive models. They are also consistent with Black and Veatch (2010) projected locations for wind facilities under a 15% renewable energy portfolio standard.

## Conservation Impacts of Wind Energy Development

What is the overlap of the areas with the highest probability of future wind energy development and those areas known to have high conservation values? To answer this question, we intersected the projected wind energy facilities with high conservation value areas. We looked at several examples from four categories of conservation value, including:

- Forest habitats
- Freshwater habitats

- Species of conservation concern
- Outdoor recreation

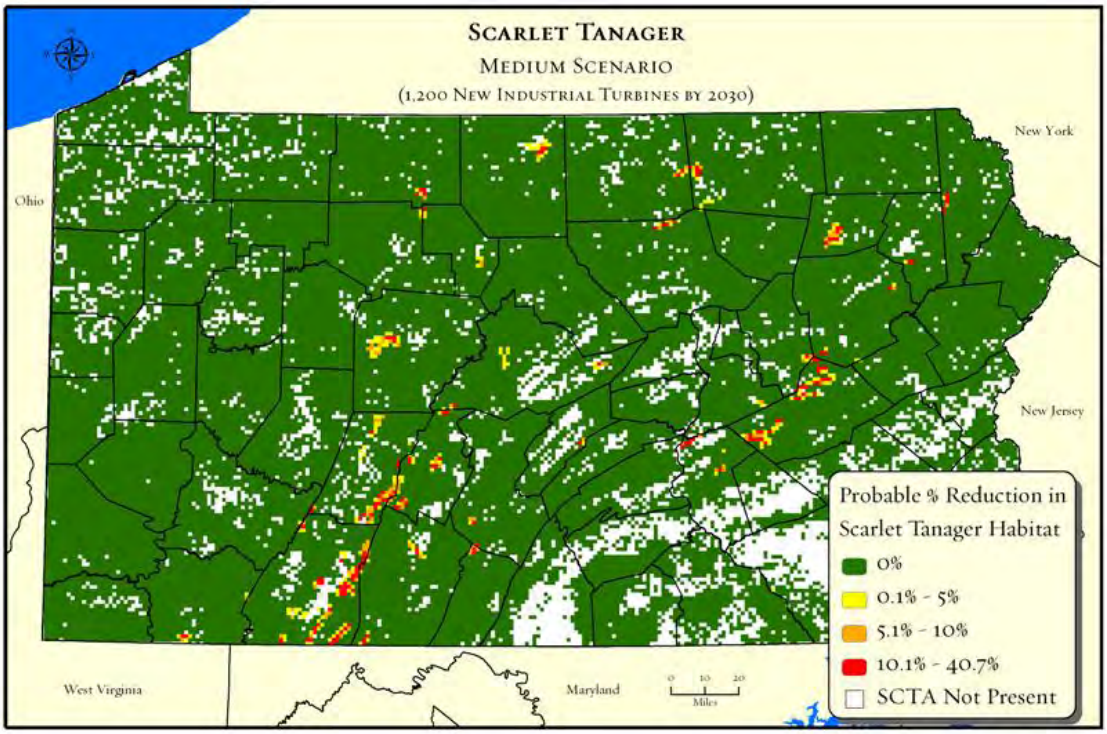
Areas of overlap between likely future wind development areas and priority conservation areas in Pennsylvania are substantially less than the conservation area overlap with likely future Marcellus development areas, largely because the projected foot print will be much smaller.

**Forests**

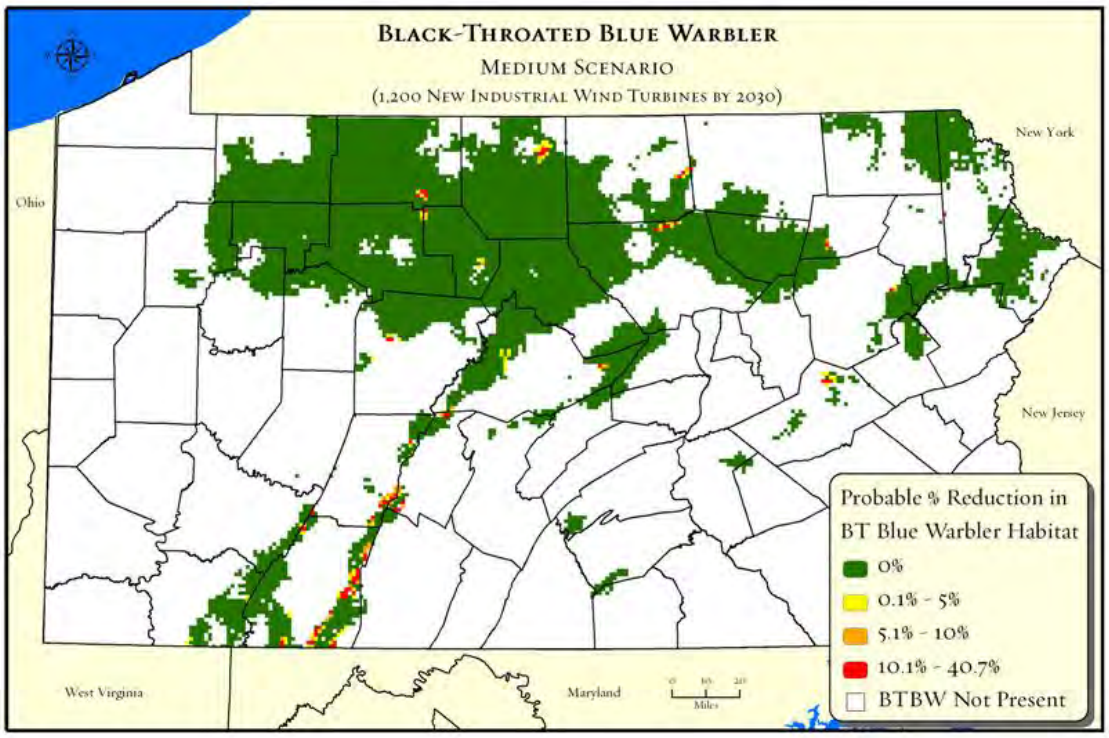
A large majority of projected wind turbines are found in forest patches, about 80 percent for each of the scenarios. The low scenario would see 600 new wind turbines in forest areas. With a cleared forest average of 1.9 acres per turbine (including roads and other infrastructure), the total forest loss would be a modest 1,900 acres. Indirect impacts to adjacent forest interior habitats would total an additional 13,400 acres. Forest impacts from the medium scenario (1,520 projected turbines in forest locations) would be 2,900 cleared forest acres and an additional 20,400 acres of adjacent forest interior habitat impacts. For the high scenario (2,720 turbines in forest areas) 5,200 acres would be cleared and an additional 36,500 acres of forest interior habitats would be affected by new adjacent clearings. On a statewide basis, the projected forest losses and accompanying interior forest habitat impacts will be minor given the Pennsylvania's 16 million acres of forest. Locally, these impacts could be significant for individual large forest patches where wind development takes place.

All forests have conservation value, but large contiguous forest patches are especially valuable because they sustain wide-ranging forest species, such as northern goshawk, than small patches. They are also more resistant to the spread of invasive species, can better withstand damage from wind and ice storms, and provide more ecosystem services – from carbon sequestration to water filtration – than small patches. The Nature Conservancy and the Western Pennsylvania Conservancy's Forest Conservation Analysis mapped nearly 25,000 forest patches in the state greater than 100 acres. Patches at least 1,000 acres in size are about a tenth of the total (2,700). The medium projected wind development scenarios indicate 73 patches (3%) greater than 1,000 acres in size are projected to have at least one wind turbine and associated infrastructure. Patches at least 5,000 acres in size are relatively rare (only 316 patches). The medium wind scenario indicates about 21 (7%) of these patches could be affected by future wind turbine development. Most affected large patches have multiple projected wind turbines (as many as 36). Typically, a large patch is split by wind development into two or three smaller patches due the linear pattern of development. Projected gas well pads, by contrast, are more likely to fragment a large patch into multiple smaller patches.

Forest interior bird species could be affected by the clearing of forest and adjacent edge effects that wind turbine facilities create in a forest context. We used data from the 2nd Breeding Bird Atlas Project (see p. 20) to assess the potential impact on forest interior species. The resulting maps show the estimated reduction in habitat for that species in each high wind development gas probability pixel (including both cleared forest and adjacent edge effects). Scarlet Tanagers are perhaps the most widespread forest interior nesting bird in the state. Since they are so widespread, the vast majority of their range in the state is outside of the most likely wind development areas. Scarlet Tanager populations could decline by an insignificant amount due to habitat losses projected in the medium scenario. Black-throated blue warblers are more narrowly distributed in Pennsylvania favoring mature northern hardwood and coniferous forests with a thick understory, frequently in mountain terrain. Likewise, population declines would also be extremely small for Black-throated blue warblers under the medium scenario.




Map showing estimated percent loss of habitat for Scarlet Tanagers under the medium wind scenario.

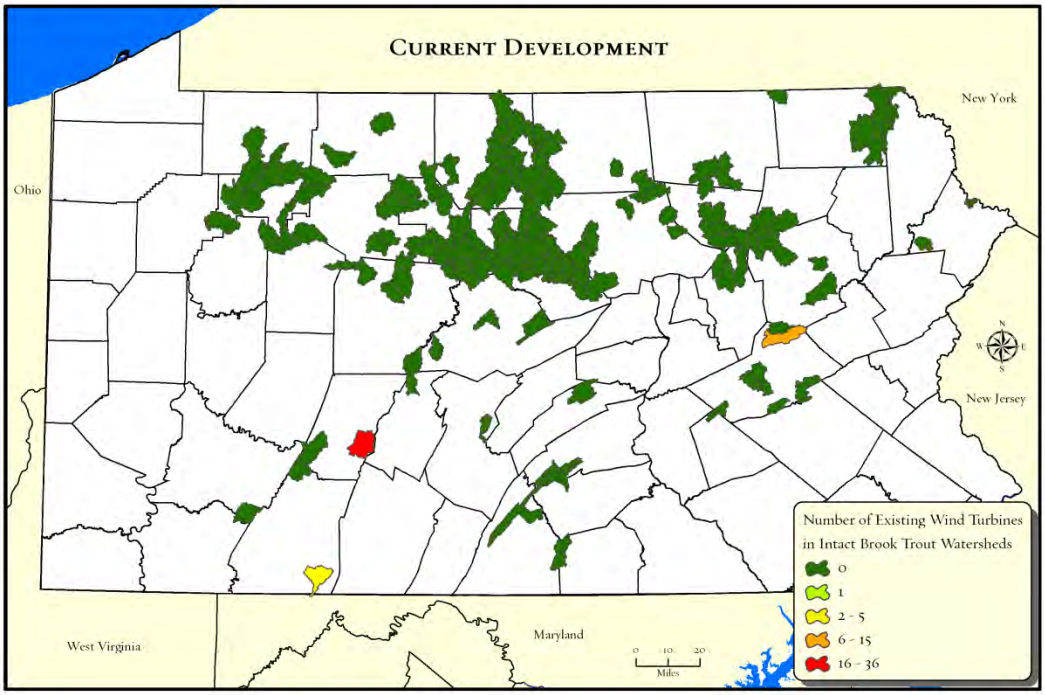



Map showing estimated percent loss of habitat for Black-Throated Blue Warblers under the medium wind scenario.

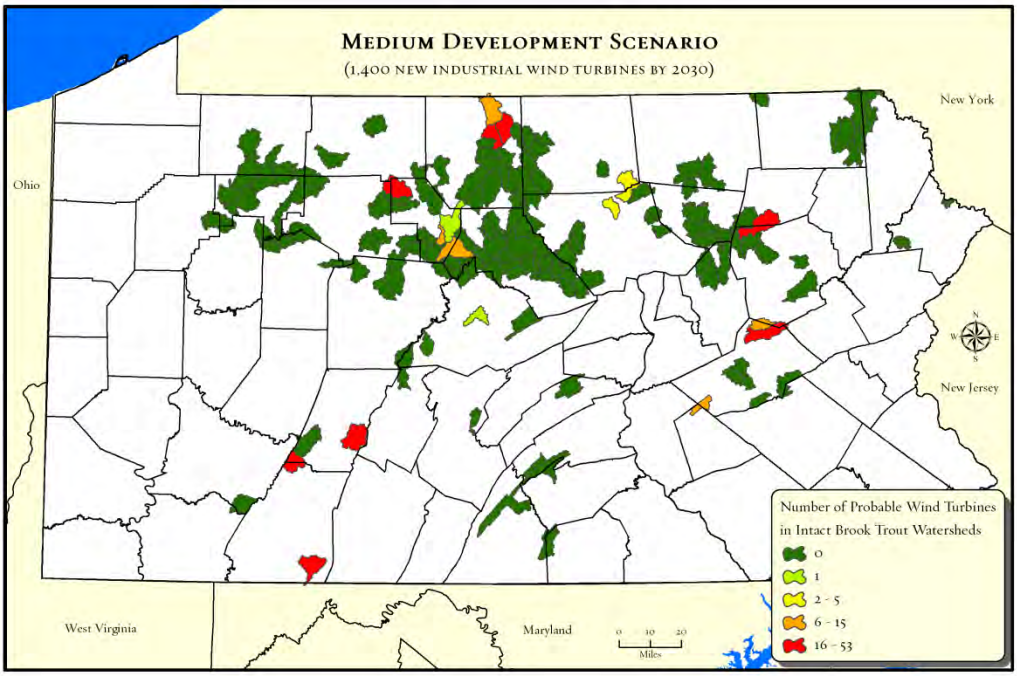
**Freshwater**

Wind energy and freshwater habitats are not often thought of in the same context since most wind facilities are generally in high elevation areas away from rivers and streams. The exceptions are small headwater streams, some of which may be classified as Exceptional Value watersheds. Our medium scenario projection indicates that 9 percent of future turbine development could be located within ½ mile of an Exceptional Value stream.

Native brook trout are one of the most sensitive species in Pennsylvania watersheds. Brook trout favor cold, highly-oxygenated water and are unusually sensitive to warmer temperatures, sediments, and contaminants. Once widely distributed across Pennsylvania, healthy populations have retreated to a shrinking number of small watersheds. The potential impact on intact brook trout watersheds, however, does increase significantly between the low to high scenarios. Wind turbines have been built in just five of the intact brook trout watersheds identified by the Eastern Brook Trout Joint Venture. That number would expand to 13 in the low scenario, 19 in the medium scenario, and 28 in the high scenario. The presence of wind turbines may pose a limited risk in many of these watersheds, principally from soil disturbance near headwater streams.




Map showing current number of wind turbines in intact and predicted intact brook trout watersheds.




Map showing projected number of wind turbines in intact brook trout watersheds (by 2030) under medium scenario.

Poorly designed or maintained sedimentation measures, especially on road cuts and stream crossings, is the principal risk to these sensitive populations.

**Rare Species**

Of the approximately 100,000 species believed to occur in Pennsylvania, just over 1 percent is tracked by The Pennsylvania Natural Heritage Program (PNHP). These species are rare, declining or otherwise considered to be of conservation concern. PNHP records indicate that 77 tracked species have populations within pixels that have a relatively high modeled probability for wind development. Most of these species are commonly found in rocky outcrops and scrub oak/pitch pine barrens habitats on ridgetops across the state. Only a handful of species, however, have more than a few occurrences overlapping with the relatively high probability wind development pixels. For example, the eastern timber rattlesnake (*Crotalus horridus*) and Allegheny woodrat (*Neotoma magister*) are strongly associated with rocky outcrops and talus slopes along or near ridgetops. Six percent of the rattlesnake's known rattlesnake breeding/denning sites and three percent of Allegheny woodrat den sites are located in relatively high wind probability pixels. The den sites are very small sites and do not include foraging areas. The Pennsylvania Natural Heritage Program has developed core habitat polygons for each Allegheny woodrat occurrence. Much larger than the den locations, these polygons indicate a much broader overlap – 43 percent – with relatively high probability pixels for wind development. The Northern long-eared Myotis bat (*Myotis septentrionalis*) has about eight percent of its known winter hibernation and summer roosting areas overlapping with relatively high probability wind development pixels. Ridgetop barrens communities in northeastern Pennsylvania have some of the state's largest concentrations of rare terrestrial species. The Nature Conservancy has mapped these communities, and some of these habitats overlap with high wind areas. In general, there appears to be relatively little overlap between tracked species occurrences in Pennsylvania and likely wind

development sites. For a handful of species, there is enough overlap to indicate the importance of surveys early in the project planning stage to identify the presence of rare species and their core habitats.

We have not addressed the potential impact of these scenarios on bird migration patterns and bat foraging populations. For more information on wind development impacts on bird and bat species, please see links to the Pennsylvania Game Commission, U.S. Fish and Wildlife Service, American Wind and Wildlife Institute, and Bat Conservation International.

**Recreation**

Wind development has not occurred on any state or federal lands in Pennsylvania to date. Since our projections assume there will not be a significant change in state land leasing policies for wind development, we have not projected new wind turbines in State Parks, State Forests or State Game Lands. Our projections, however, do indicate that wind turbines will be located in close proximity (sometimes as close as 500 feet) to many state lands. They are likely to be highly visible in some heavily visited areas, such as Blue Knob State Park in Bedford County, where natural landscape vistas are a prime attraction.

## Key Findings

Key findings from the Pennsylvania Energy Impacts Assessment include:

- Projections of between 750 and 2,900 new wind turbines developed on ridgetops and high plateaus by 2030, depending on the size of the Pennsylvania Alternative Energy Portfolio standard. There are currently an estimated 500 wind turbines built in the state.

- Wind turbine facilities are likely to be developed in half of the state's counties, especially along the Allegheny front in western Pennsylvania and on high Central Appalachian ridges in central and northeastern parts of the state;

- Nearly eighty percent of turbine locations are projected to be in forest areas, with forest clearing projected to range between 1,900 and 5,200 acres depending on the number of turbines developed. An additional range of 13,400 to 36,500 acres of forest interior habitat impacts are projected due to new edges created by turbine pads and roads;

- On a statewide basis, the projected forest clearing from turbine development is relatively minor, though some of the state's largest forest patches (>5,000 acres) could be fragmented into smaller patches by projected wind turbine development;

- Impacts on forest interior breeding bird habitats appear to be limited, largely because the overall footprint for the projected wind turbine facilities is small in comparison to the typical breeding range of these species in Pennsylvania. The study did not assess impacts to migratory pathways for birds or foraging bats.

- Relatively few watersheds ranked as "intact" by the Eastern Brook Trout Joint Venture are affected by projected wind turbine development. Several intact watersheds, however, could see several dozen wind turbines. In a number of cases, these small watersheds are projected to see significant Marcellus gas development as well. Given the cumulative impact of these activities, rigorously designed and monitored sediment control measures will be needed to protect sensitive brook trout populations.

- A relatively small handful of rare species occurrences tracked by the Pennsylvania Natural Heritage Program are found in areas with high probability for wind development. These species tend to be associated with rocky outcrops and barrens communities typically found on ridge tops, including the Allegheny wood rat, the eastern timber rattlesnake, and the northern long-eared Myotis bat.

- Wind development is not projected to occur on Pennsylvania's public lands. Existing and projected wind turbines, however, will be close to some of Pennsylvania's most heavily visited outdoor recreation areas where scenic natural vistas are a major attraction.

## Additional Information

- American Wind Energy Association (2010). U.S. Wind Projects Database. http://www.awea.org/la_usprojects.cfm

- Black and Veatch (2010). Assessment of a 15 Percent Pennsylvania Alternative Energy Portfolio Standard. Report prepared for the Community Foundation for the Alleghenies, Johnstown, PA. http://www.cfalleghenies.org/pdf/aepss.pdf

- Federal Aviation Administration (FAA) permits for wind turbines: https://oeaaa.faa.gov/oeaaa/external/public/publicAction.jsp?action=showCaseDownloadForm

- Federal Aviation Administration (FAA), Obstruction Evaluation / Airport Airspace Analysis (OE/AAA): https://oeaaa.faa.gov/oeaaa/external/public/publicAction.jsp?action=showCaseDownloadForm

- Pennsylvania Wind Farms and Wildlife Collaborative: http://www.dcnr.state.pa.us/wind/index.aspx

- PA Game Commission (2007) Wind Energy Voluntary Cooperative Agreement and First Annual Report for the Wind Energy Voluntary Cooperative Agreement: http://www.portal.state.pa.us/portal/server.pt?open=514&objID=613068&mode=2

- Pennsylvania Department of Environmental Protection, Chapter 93 Water Quality Standards, Exceptional Value and High Quality Streams: data downloaded from Pennsylvania Spatial Data Access: (www.pasda.psu.edu)

- U.S. Department of Energy TrueWind 80 Meter Wind Resource Maps: http://www.windpoweringamerica.gov/wind_maps.asp

- U.S. Fish and Wildlife Service Wind Turbine Advisory Committee: http://www.fws.gov/habitatconservation/windpower/wind_turbine_advisory_committee.html

- U.S. Environmental Protection Agency summary of forest fragmentation effects: http://cfpub.epa.gov/eroe/index.cfm?fuseaction=detail.viewInd&lv=list.listByAlpha&r=219658&subtop=210

- Overview of forest fragmentation impacts on forest interior nesting species: http://www.state.nj.us/dep/fgw/neomigr.htm

- Overview of Pennsylvania High Quality and Exceptional Value Streams: http://www.dcnr.state.pa.us/wlhabitat/aquatic/streamdist.aspx

- Eastern Brook Trout Joint Venture intact brook trout watersheds: http://128.118.47.58/EBTJV/ebtjv2.html

- Overview of Carnegie Museum of Natural History, Powdermill Nature Reserve, and the Pennsylvania Game Commission's 2nd Pennsylvania Breeding Bird Atlas Project: http://www.carnegiemnh.org/powdermill/atlas/2pbba.html

- Pennsylvania Natural Heritage Program, including lists of globally rare and state endangered and imperiled species: http://www.naturalheritage.state.pa.us/

- U.S. Department of Agriculture, Natural Resources Conservation Service, National Agriculture Imagery Program: http://datagateway.nrcs.usda.gov/GDGOrder.aspx

pennsylvania
DEPARTMENT OF CONSERVATION
AND NATURAL RESOURCES

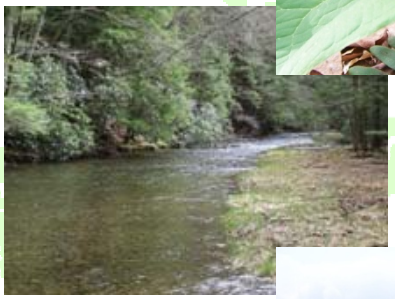
# Impacts of Leasing Additional State Forest for Natural Gas Development

pennsylvania
DEPARTMENT OF CONSERVATION
AND NATURAL RESOURCES

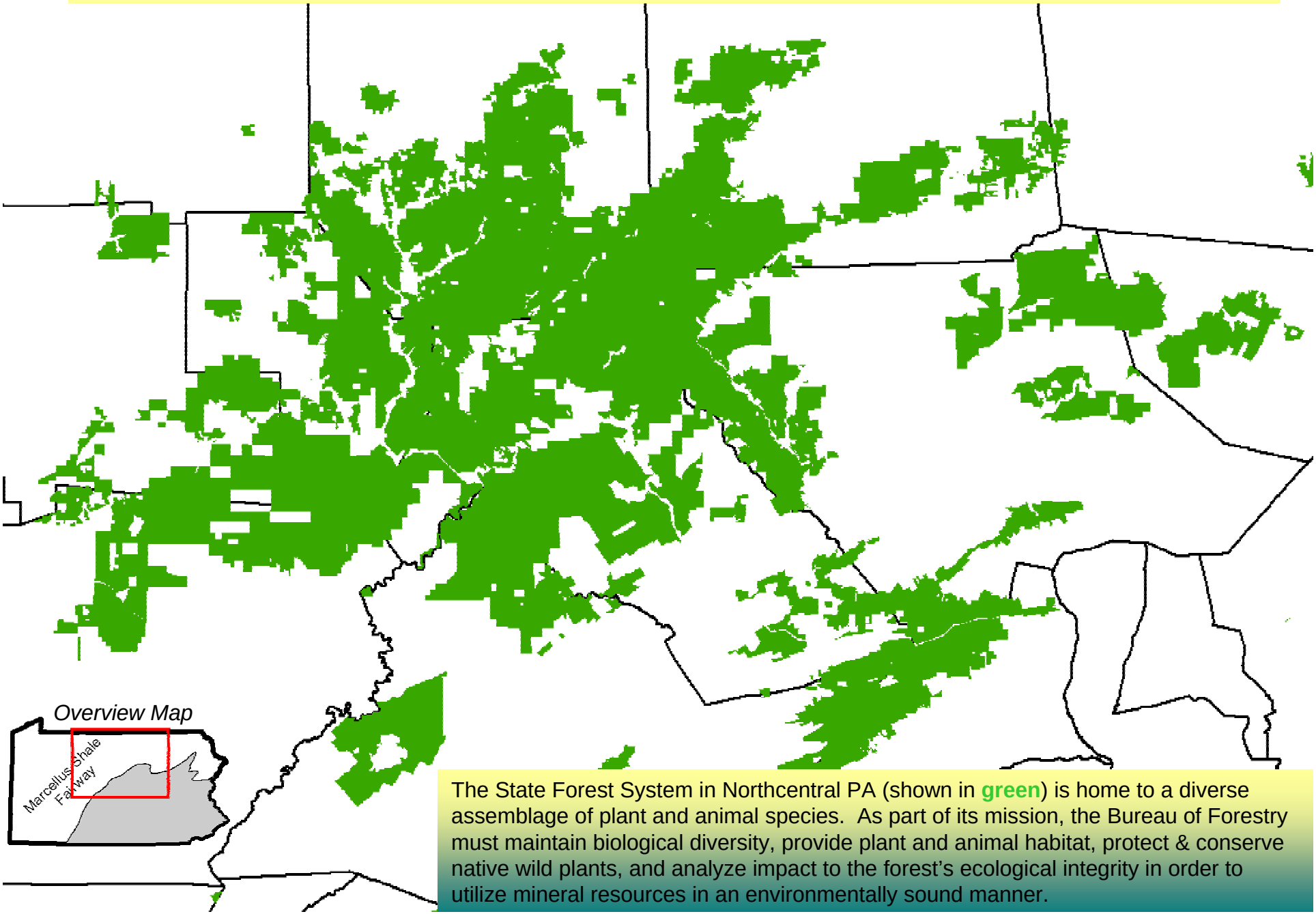
DCNR is entrusted to balance the uses and values of our state forests while protecting the integrity and health of the whole system.

There are proposals and public debate about the merits of a moratorium on natural gas drilling on state forest.

This mapping analysis demonstrates how any additional leasing involving surface disturbance upsets the sustainable balance DCNR is charged to maintain.

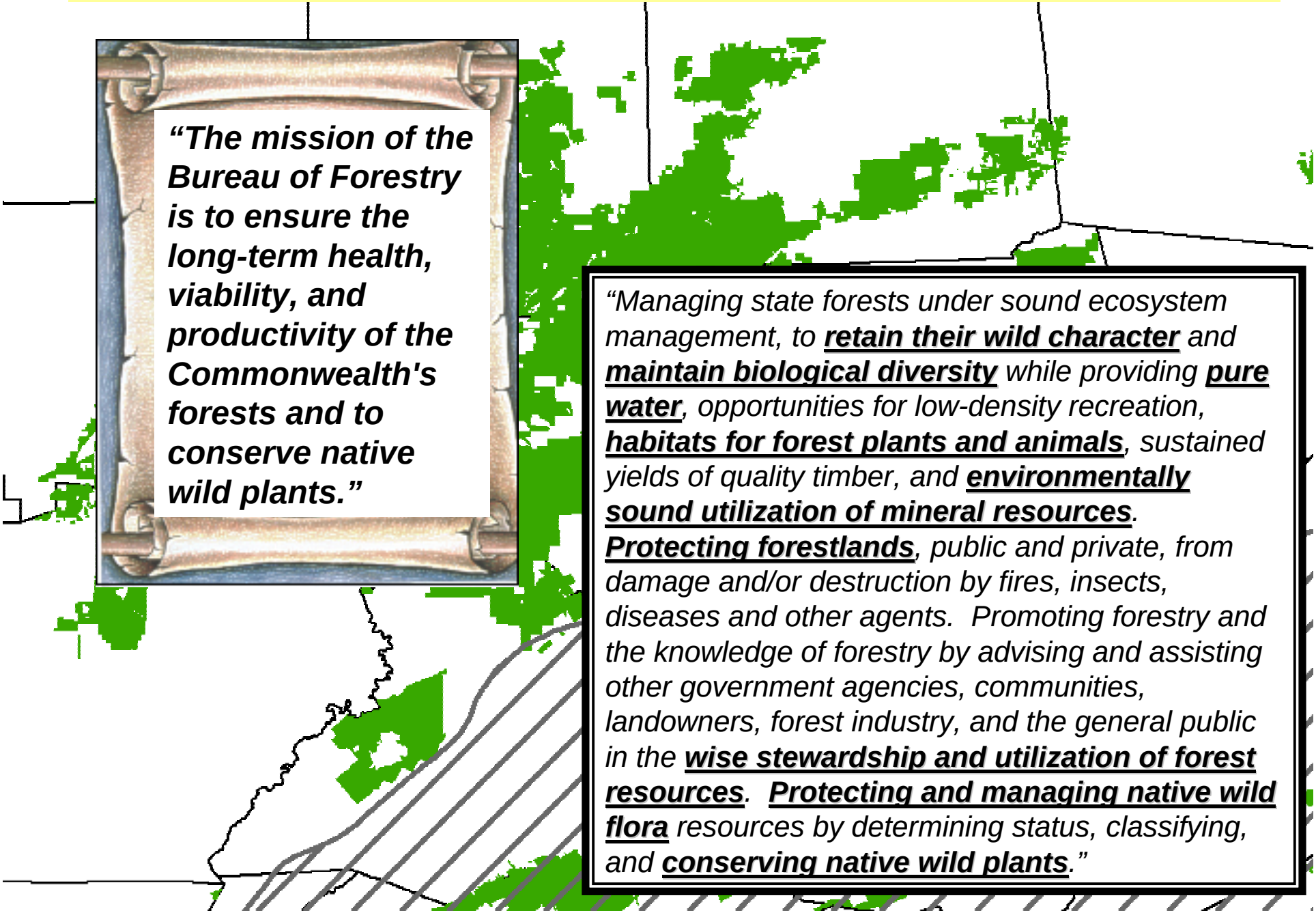


# State Forest Land in North-Central Pennsylvania




The State Forest System in Northcentral PA (shown in **green**) is home to a diverse assemblage of plant and animal species. As part of its mission, the Bureau of Forestry must maintain biological diversity, provide plant and animal habitat, protect & conserve native wild plants, and analyze impact to the forest's ecological integrity in order to utilize mineral resources in an environmentally sound manner.

# State Forest Land in Northcentral Pennsylvania



***"The mission of the Bureau of Forestry is to ensure the long-term health, viability, and productivity of the Commonwealth's forests and to conserve native wild plants."***

*"Managing state forests under sound ecosystem management, to* ***retain their wild character*** *and* ***maintain biological diversity*** *while providing* ***pure water****, opportunities for low-density recreation,* ***habitats for forest plants and animals****, sustained yields of quality timber, and* ***environmentally sound utilization of mineral resources****.* ***Protecting forestlands****, public and private, from damage and/or destruction by fires, insects, diseases and other agents. Promoting forestry and the knowledge of forestry by advising and assisting other government agencies, communities, landowners, forest industry, and the general public in the* ***wise stewardship and utilization of forest resources****.* ***Protecting and managing native wild flora*** *resources by determining status, classifying, and* ***conserving native wild plants****."*

# Maintaining the Forest's Ecological Integrity

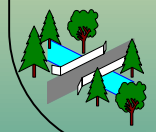
## Species of Concern

Native Biological Diversity
Threatened / Endangered Spp.
Rare / Declining Spp.

## Unique Areas

Wild & Natural Areas
Steep, Wet, & Rocky Areas
Old Growth
Wild Plant Sanctuaries

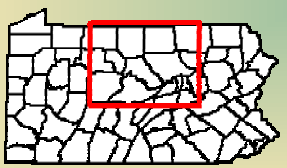
## Road, Trail, & Stream Buffers

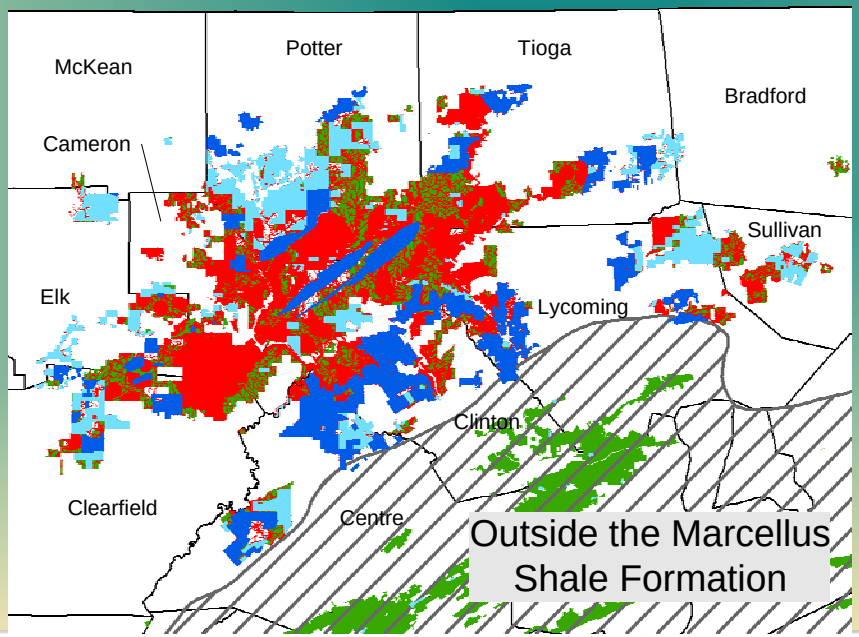


Aesthetics / Scenery
Corridors
Connectivity
Water Quality

**Legend**

- DCNR Gas Lease
- Severed Rights
- Ecologically Sensitive
- Other State Forest






State Forest Land in the Marcellus Shale Region

State Forest Land Currently Leased or Severed

1,500,000 acres

Unleased Land in Ecologically Sensitive Areas

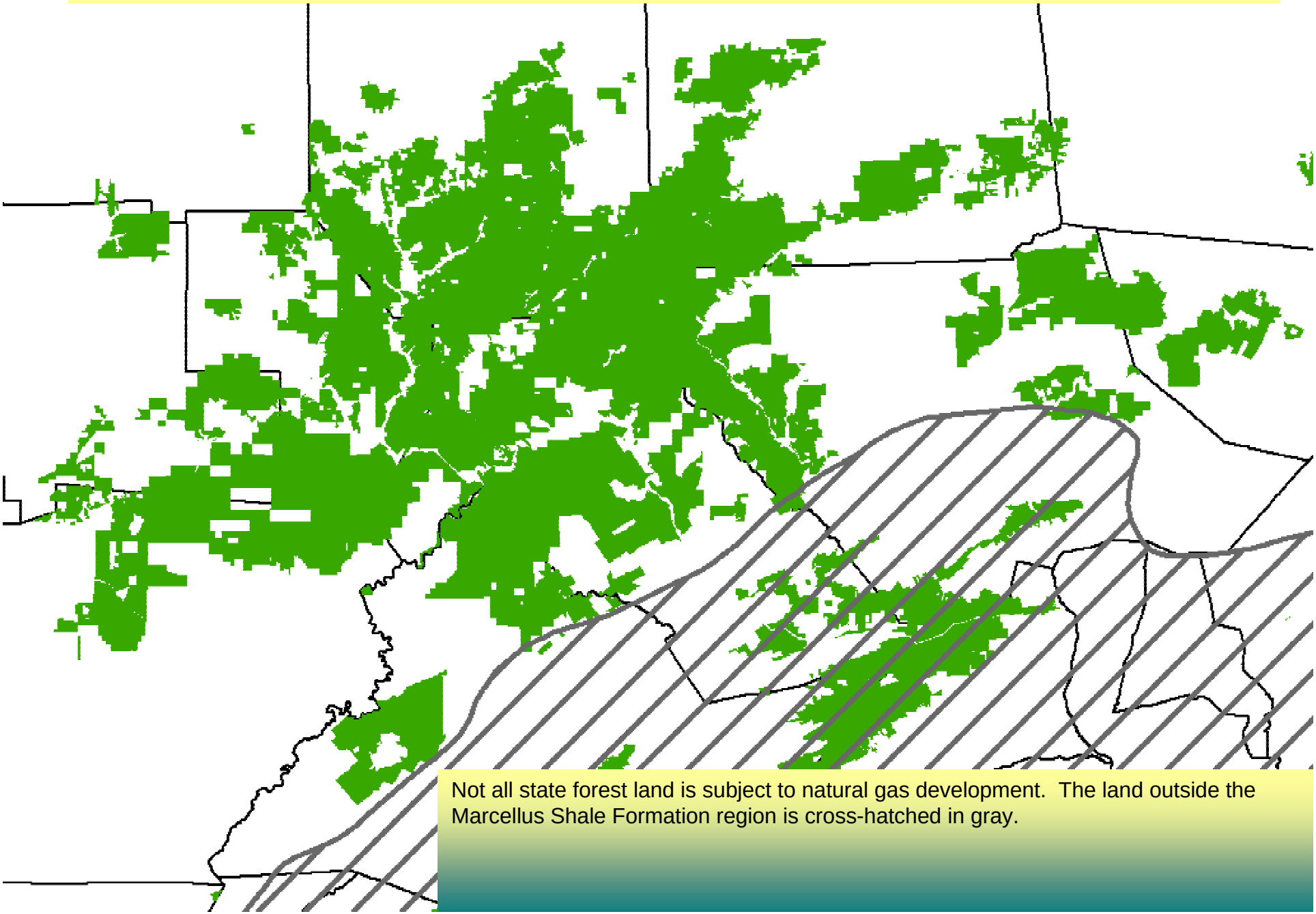
- 700,000 acres

Inaccessible w/o Damaging Ecologically Sensitive Areas

- 702,500 acres

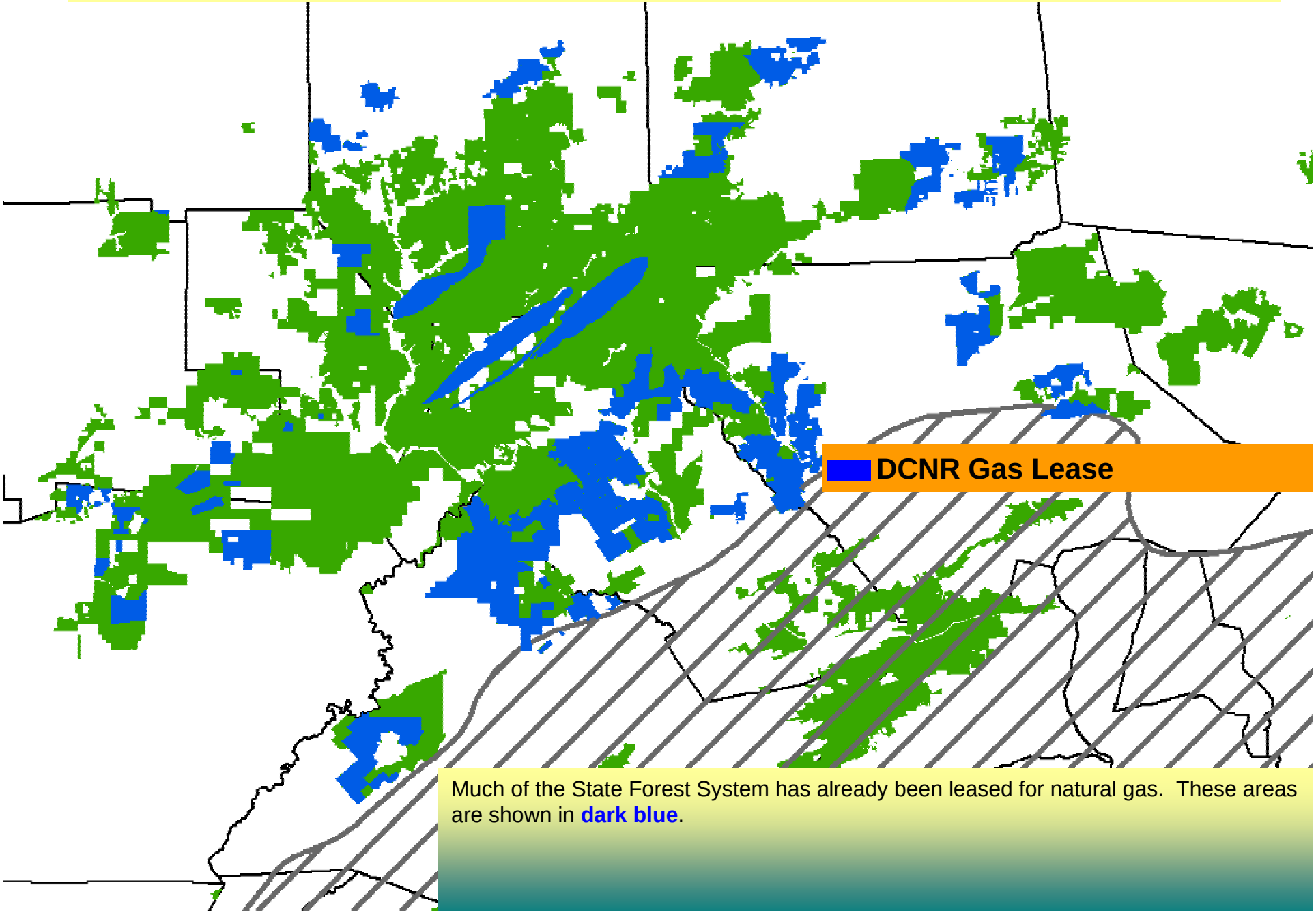
**97,500** acres

# Maintaining the Forest's Ecological Integrity



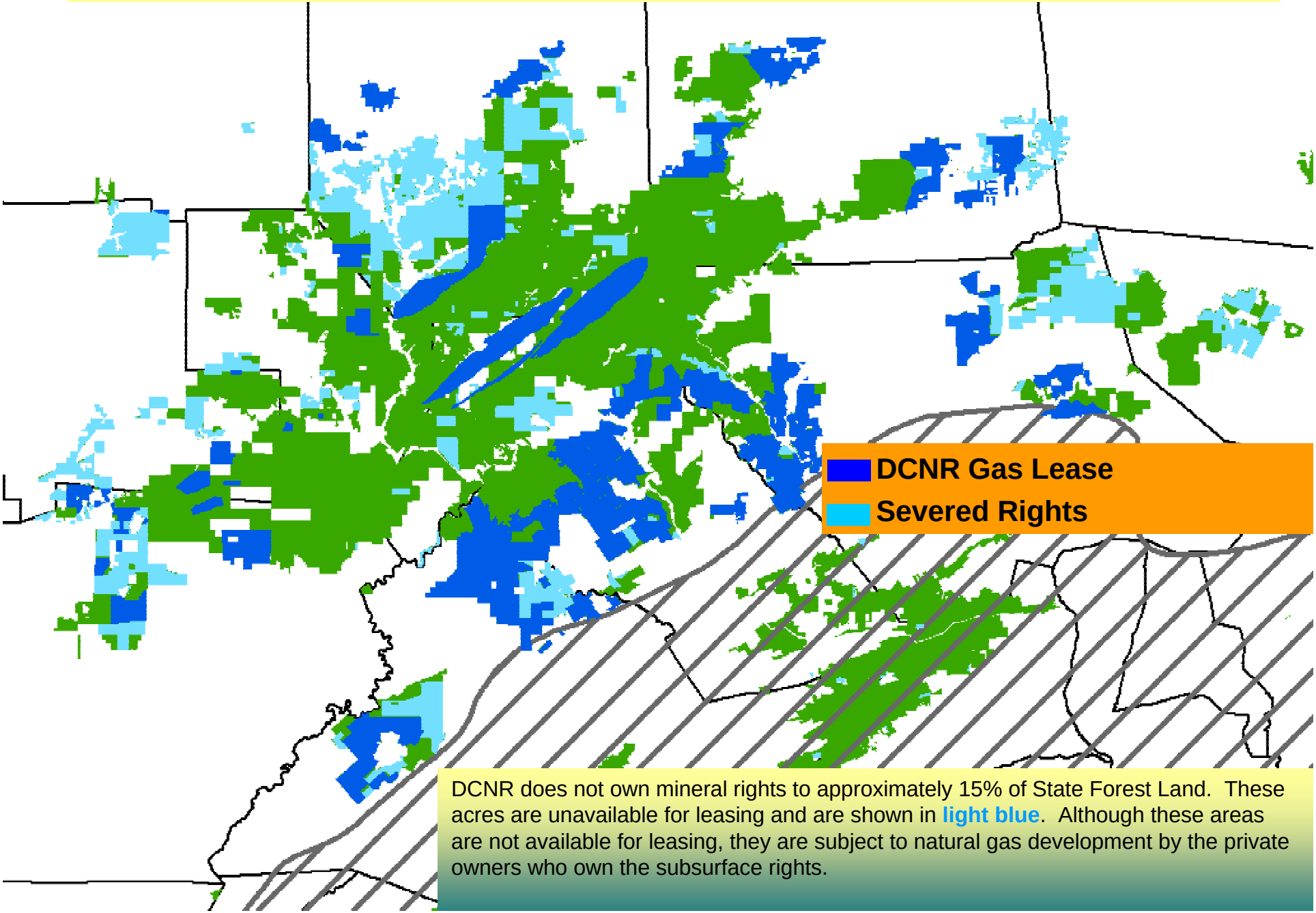
Not all state forest land is subject to natural gas development. The land outside the Marcellus Shale Formation region is cross-hatched in gray.

# Maintaining the Forest's Ecological Integrity



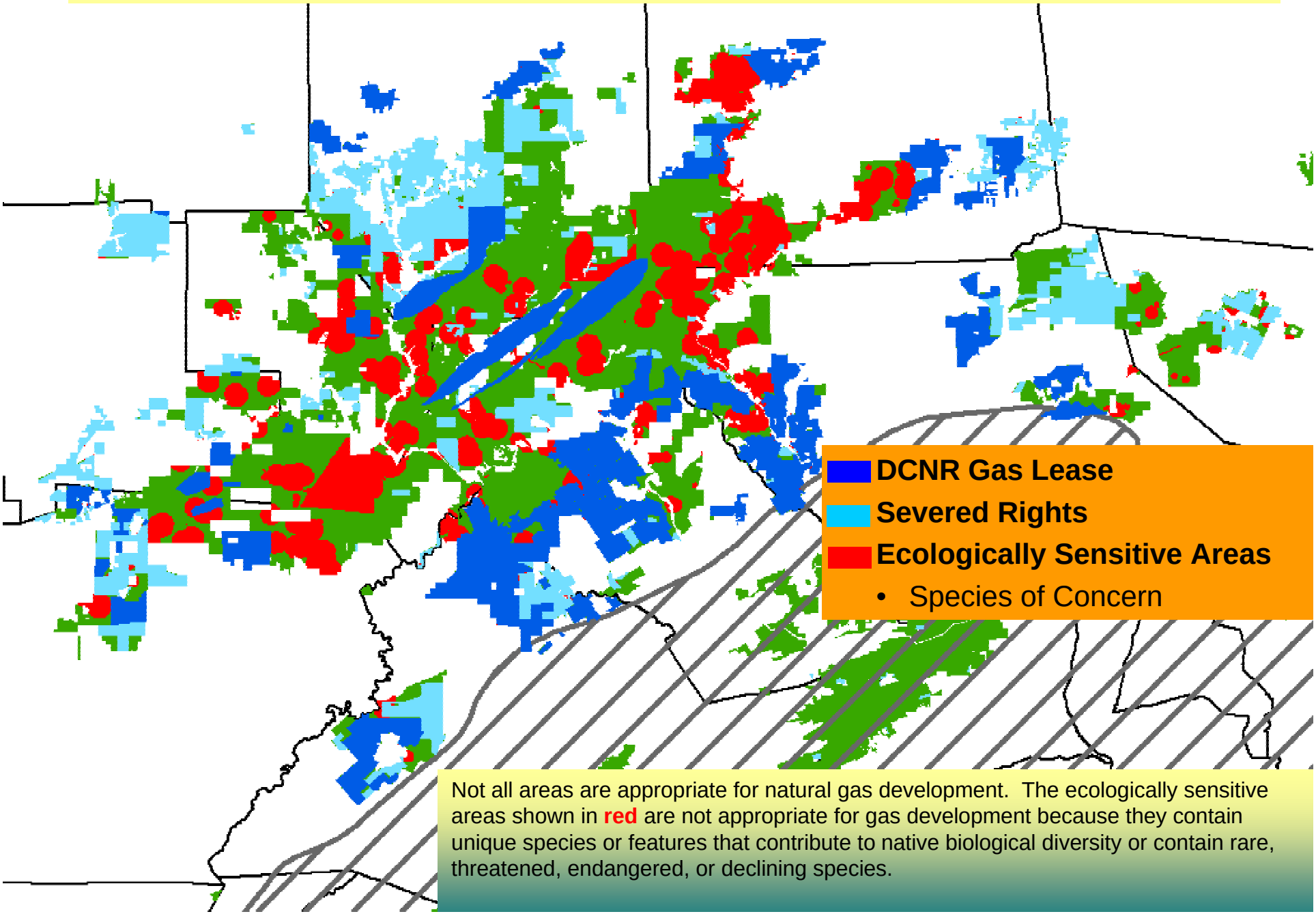

Much of the State Forest System has already been leased for natural gas. These areas are shown in **dark blue**.

# Maintaining the Forest's Ecological Integrity



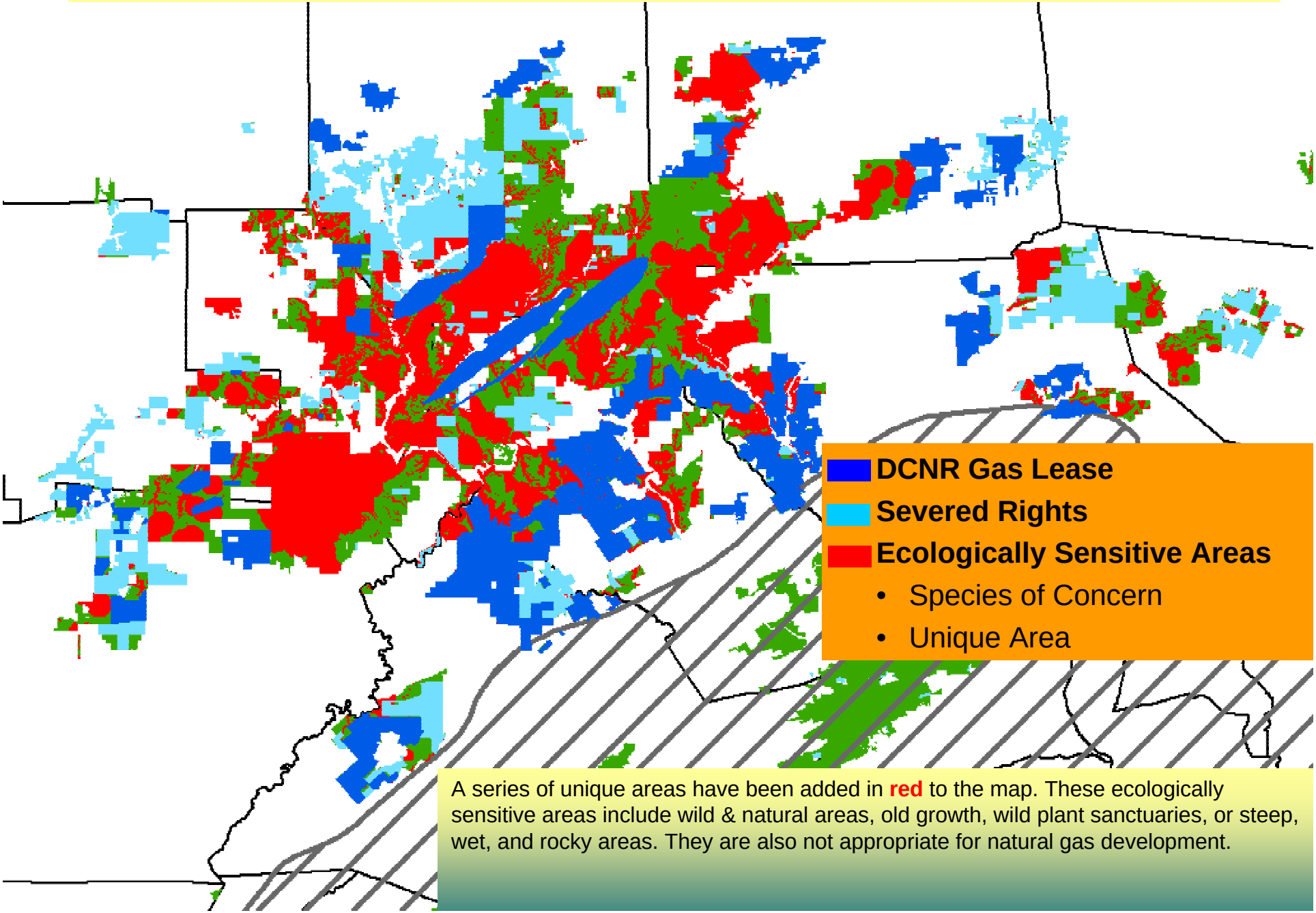

DCNR does not own mineral rights to approximately 15% of State Forest Land. These acres are unavailable for leasing and are shown in **light blue**. Although these areas are not available for leasing, they are subject to natural gas development by the private owners who own the subsurface rights.

# Maintaining the Forest's Ecological Integrity



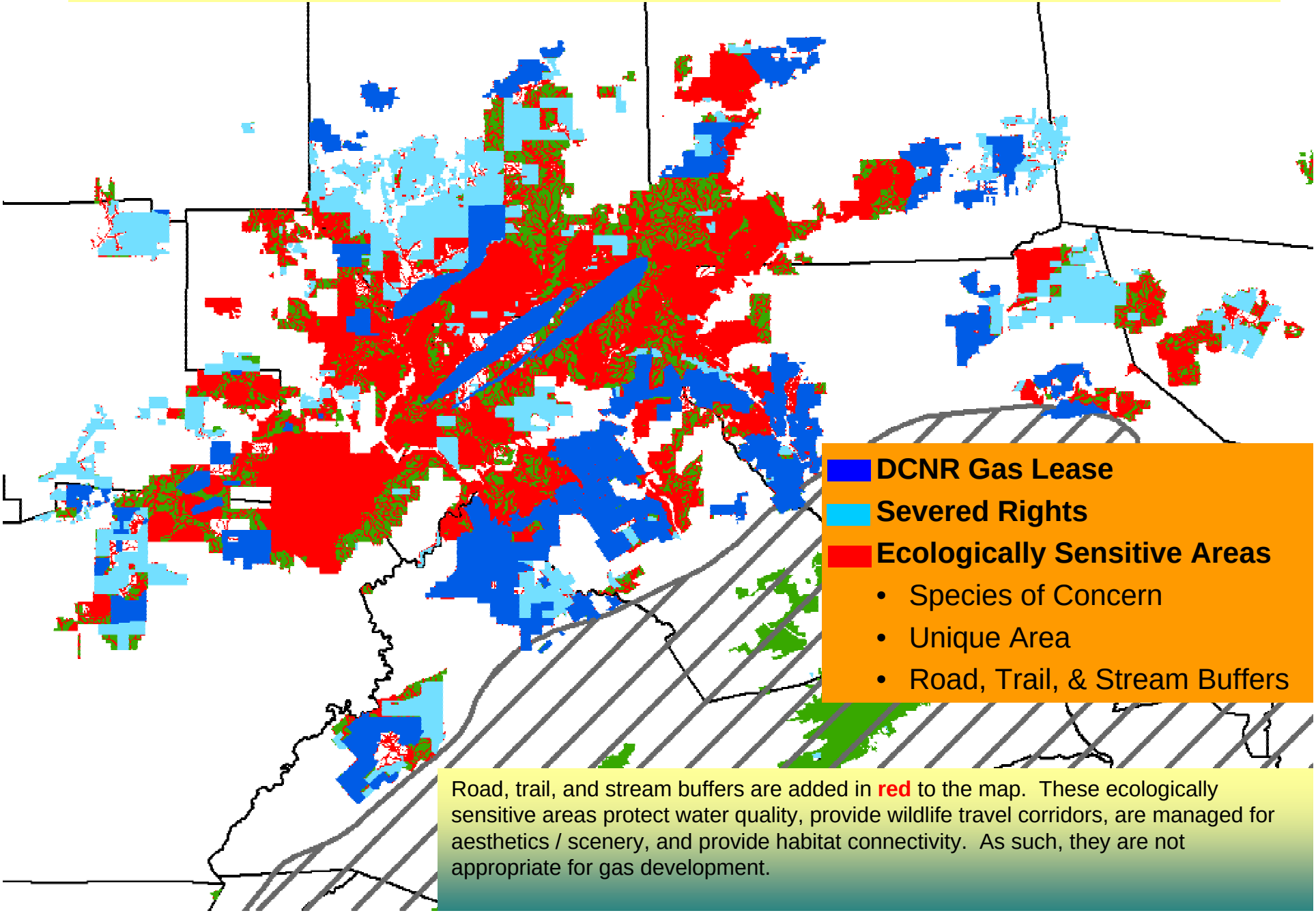

Not all areas are appropriate for natural gas development. The ecologically sensitive areas shown in **red** are not appropriate for gas development because they contain unique species or features that contribute to native biological diversity or contain rare, threatened, endangered, or declining species.

# Maintaining the Forest's Ecological Integrity



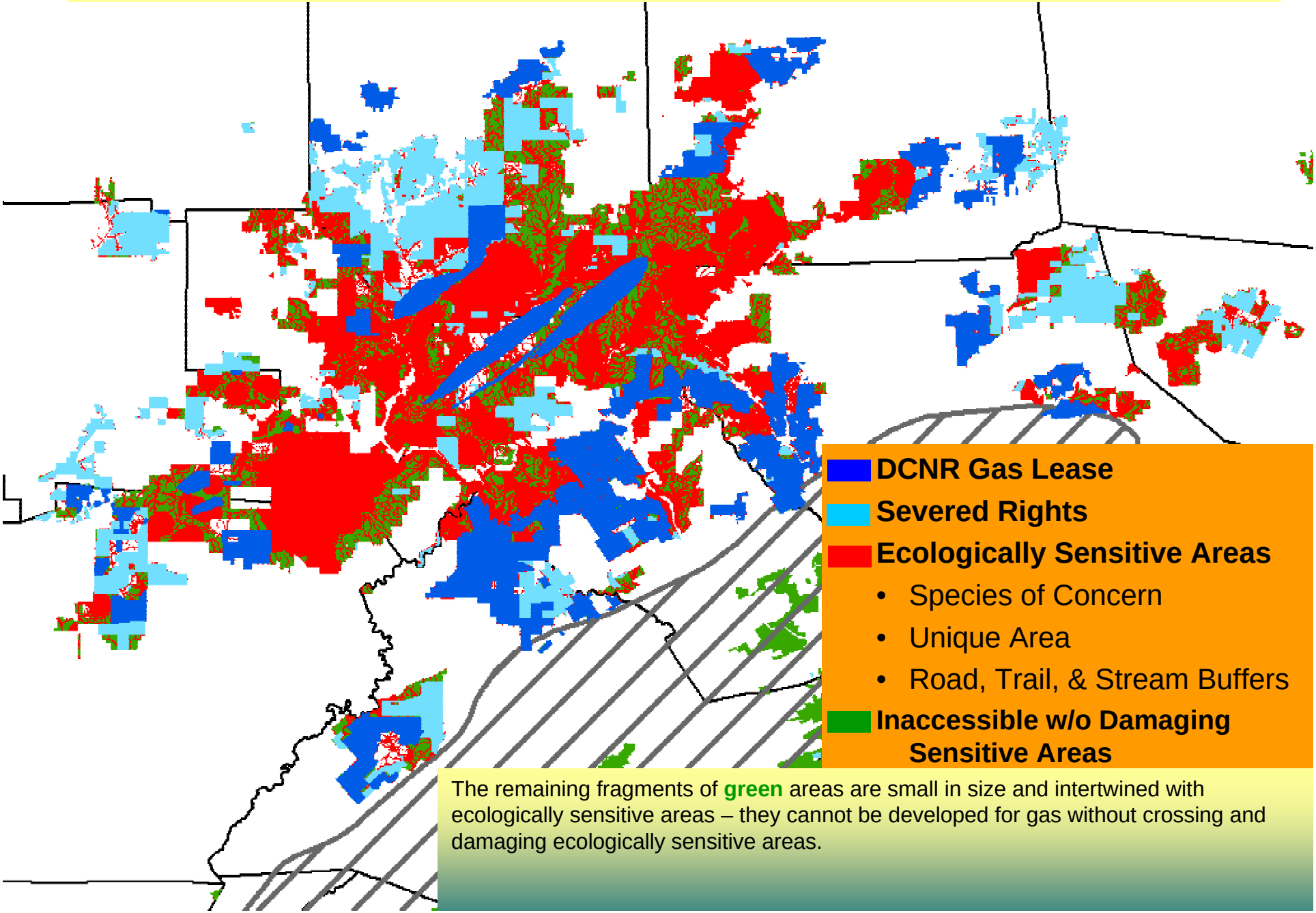

A series of unique areas have been added in **red** to the map. These ecologically sensitive areas include wild & natural areas, old growth, wild plant sanctuaries, or steep, wet, and rocky areas. They are also not appropriate for natural gas development.

# Maintaining the Forest's Ecological Integrity




Road, trail, and stream buffers are added in **red** to the map. These ecologically sensitive areas protect water quality, provide wildlife travel corridors, are managed for aesthetics / scenery, and provide habitat connectivity. As such, they are not appropriate for gas development.

# Maintaining the Forest's Ecological Integrity




The remaining fragments of **green** areas are small in size and intertwined with ecologically sensitive areas – they cannot be developed for gas without crossing and damaging ecologically sensitive areas.

# Maintaining the Forest's Wild Character

No Management

## Primitive

- Peace, Solitude, Remoteness, Backland Experience
- Non-Motorized Recreation

298,093 acres

## Semi-Primitive

- Moderate Probability of Experiencing Solitude
- Limited Timber & Gas Activities

412,806 acres

## Semi-Developed

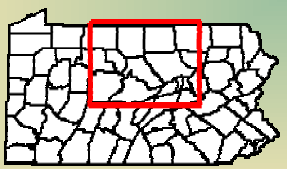
- Commonly Encounter Other Recreationists
- Motorized Recreation
- Timber & Gas Activities

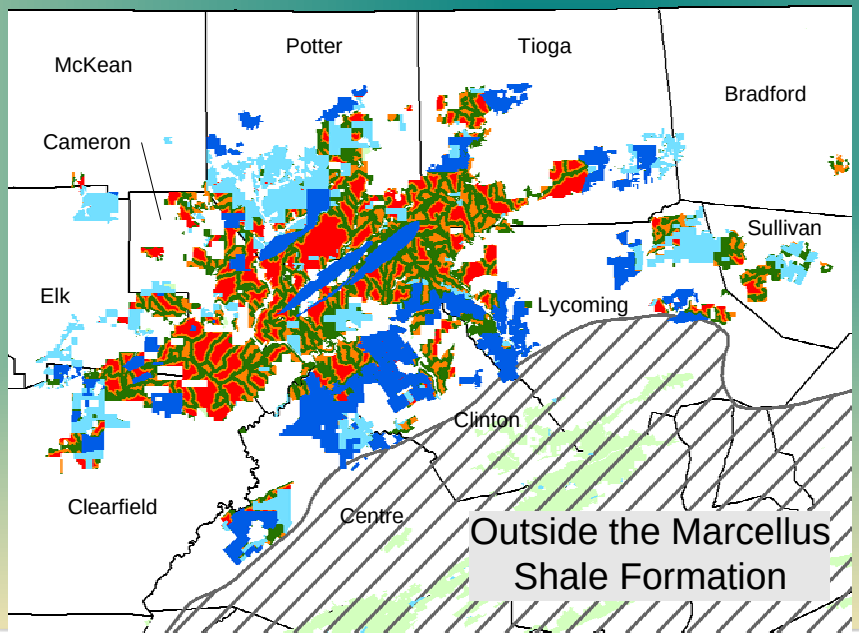
706,412 acres

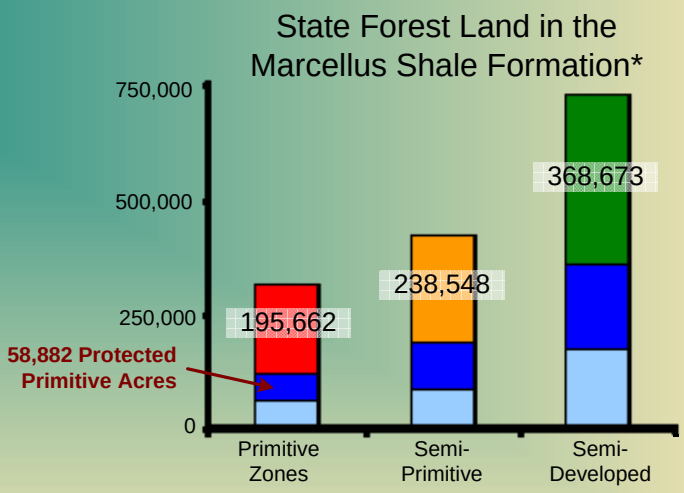
Intense Management

**Legend**

- DCNR Gas Lease
- Severed Rights
- Semi-Developed
- Semi-Primitive
- Off Limits (Primitive)

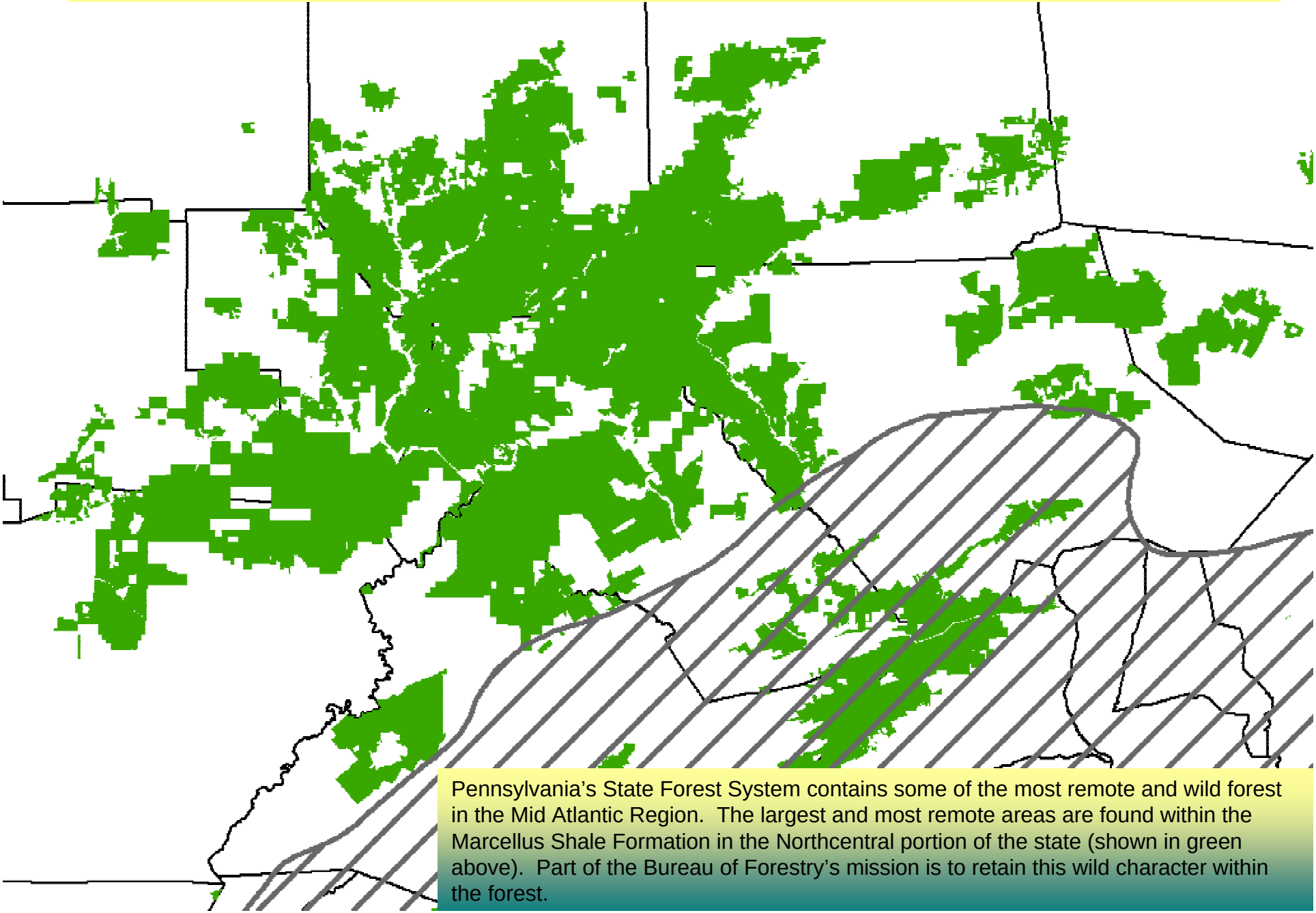




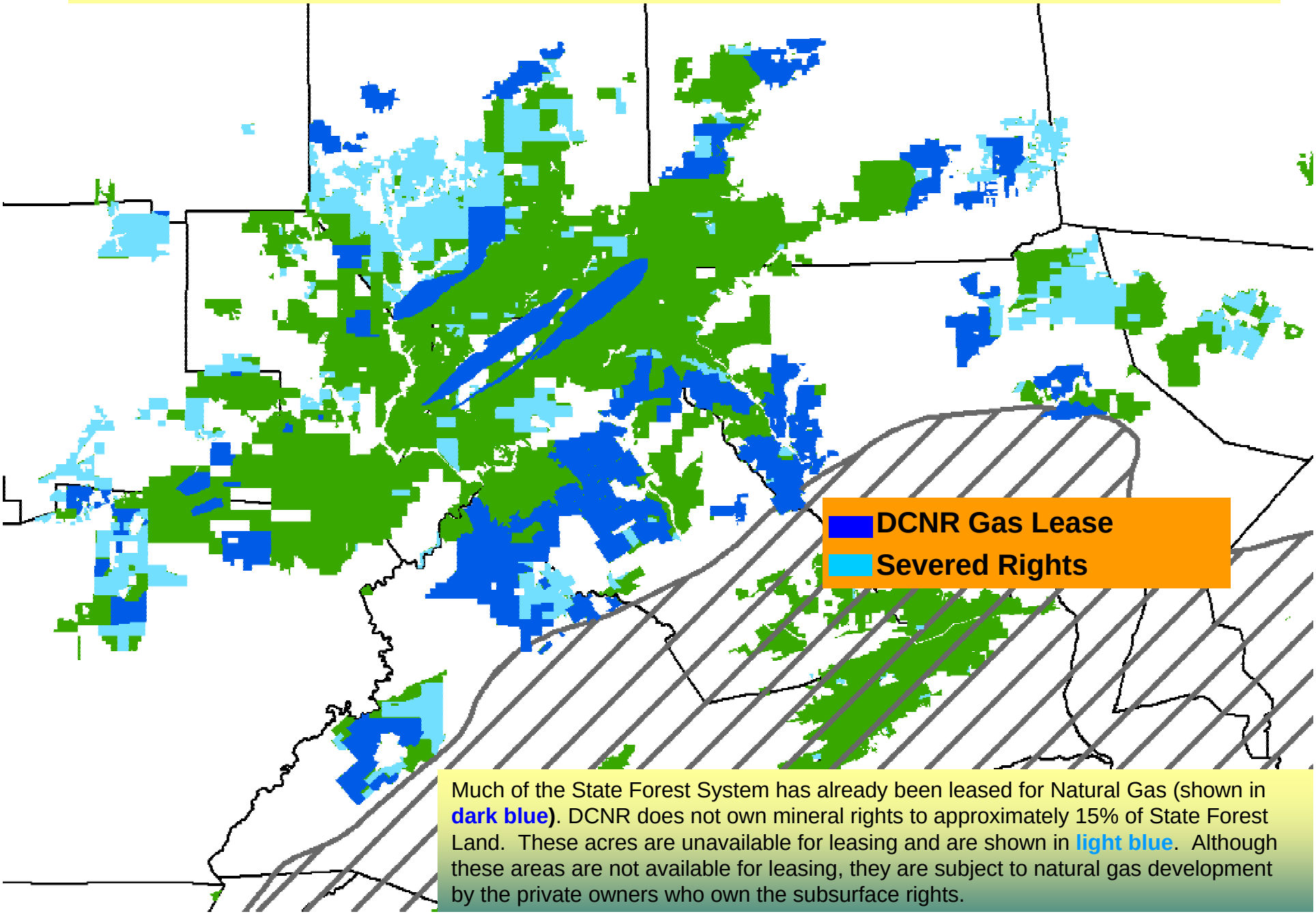

*Delaware State Forest not included.

# Maintaining the Forest's Wild Character



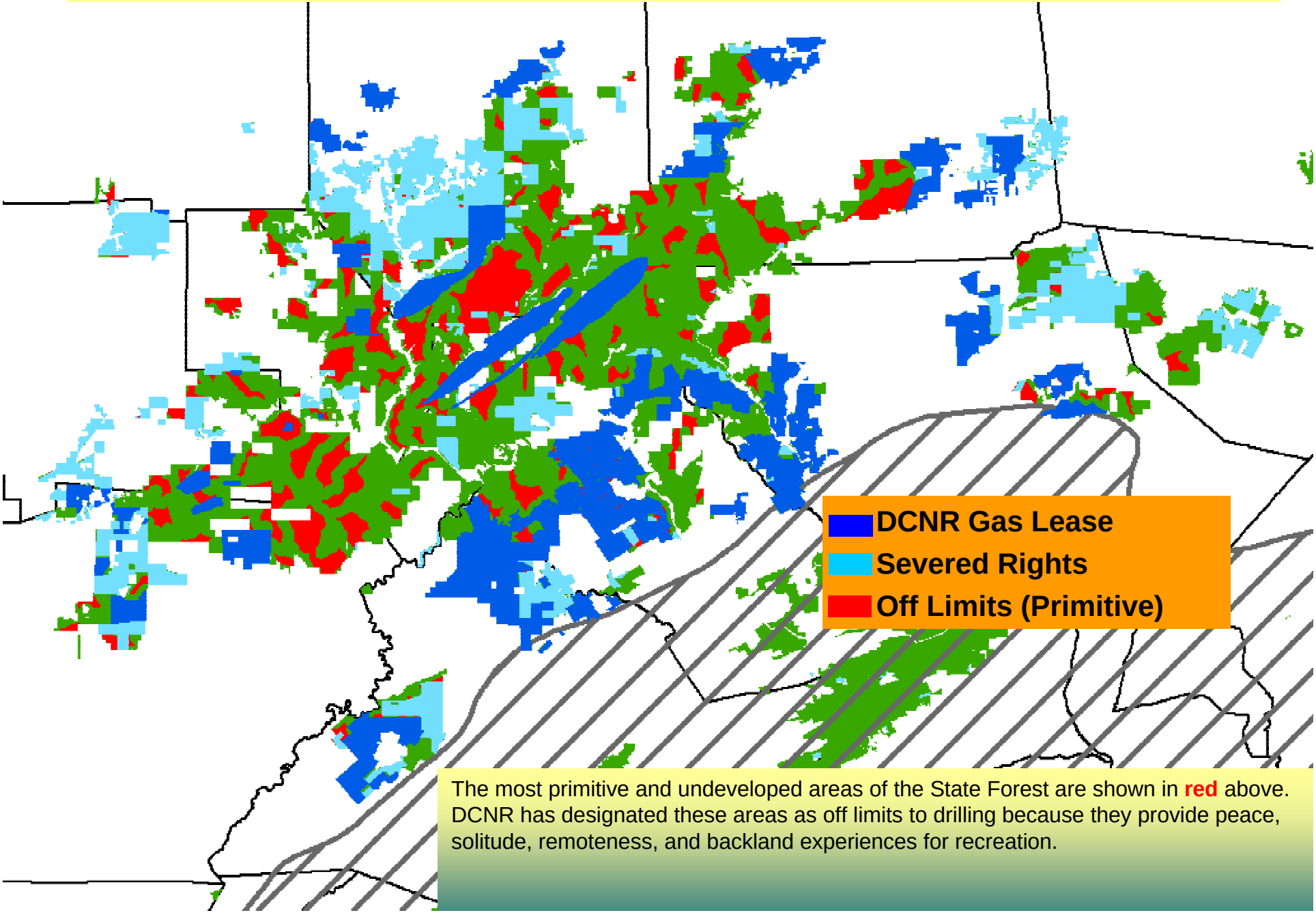
Pennsylvania's State Forest System contains some of the most remote and wild forest in the Mid Atlantic Region. The largest and most remote areas are found within the Marcellus Shale Formation in the Northcentral portion of the state (shown in green above). Part of the Bureau of Forestry's mission is to retain this wild character within the forest.

# Maintaining the Forest's Wild Character



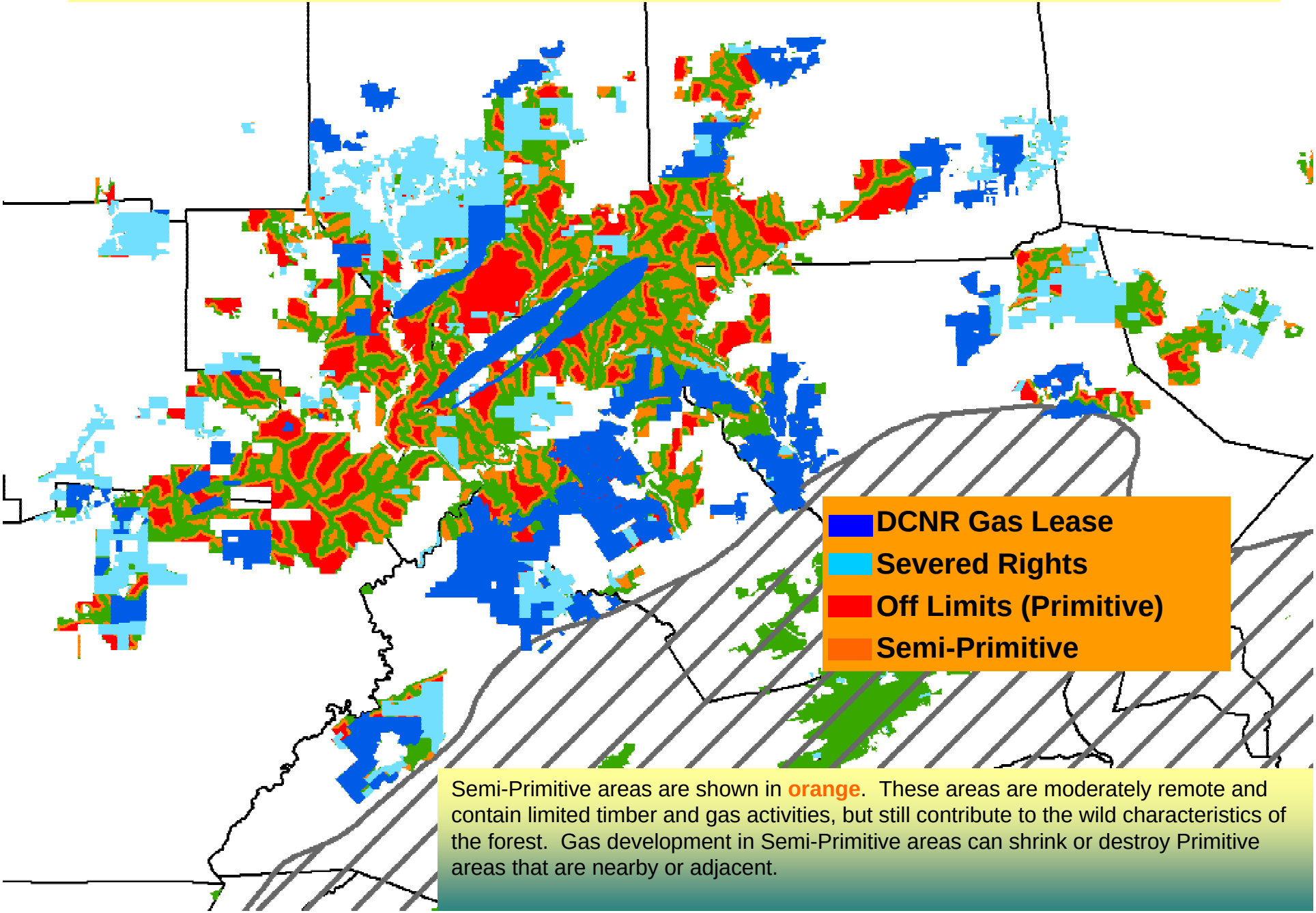

Much of the State Forest System has already been leased for Natural Gas (shown in **dark blue**). DCNR does not own mineral rights to approximately 15% of State Forest Land. These acres are unavailable for leasing and are shown in **light blue**. Although these areas are not available for leasing, they are subject to natural gas development by the private owners who own the subsurface rights.

# Maintaining the Forest's Wild Character



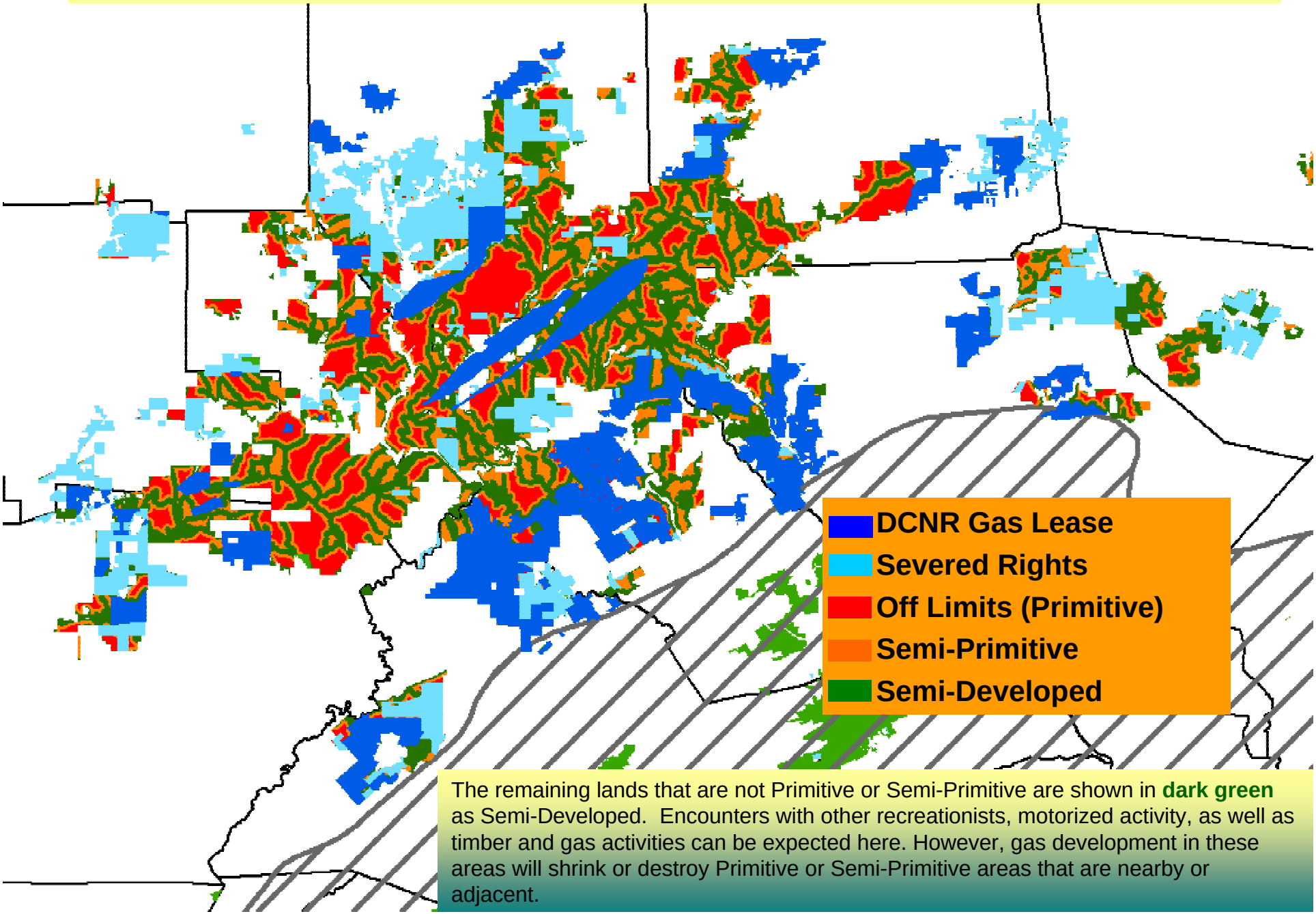

The most primitive and undeveloped areas of the State Forest are shown in **red** above. DCNR has designated these areas as off limits to drilling because they provide peace, solitude, remoteness, and backland experiences for recreation.

# Maintaining the Forest's Wild Character



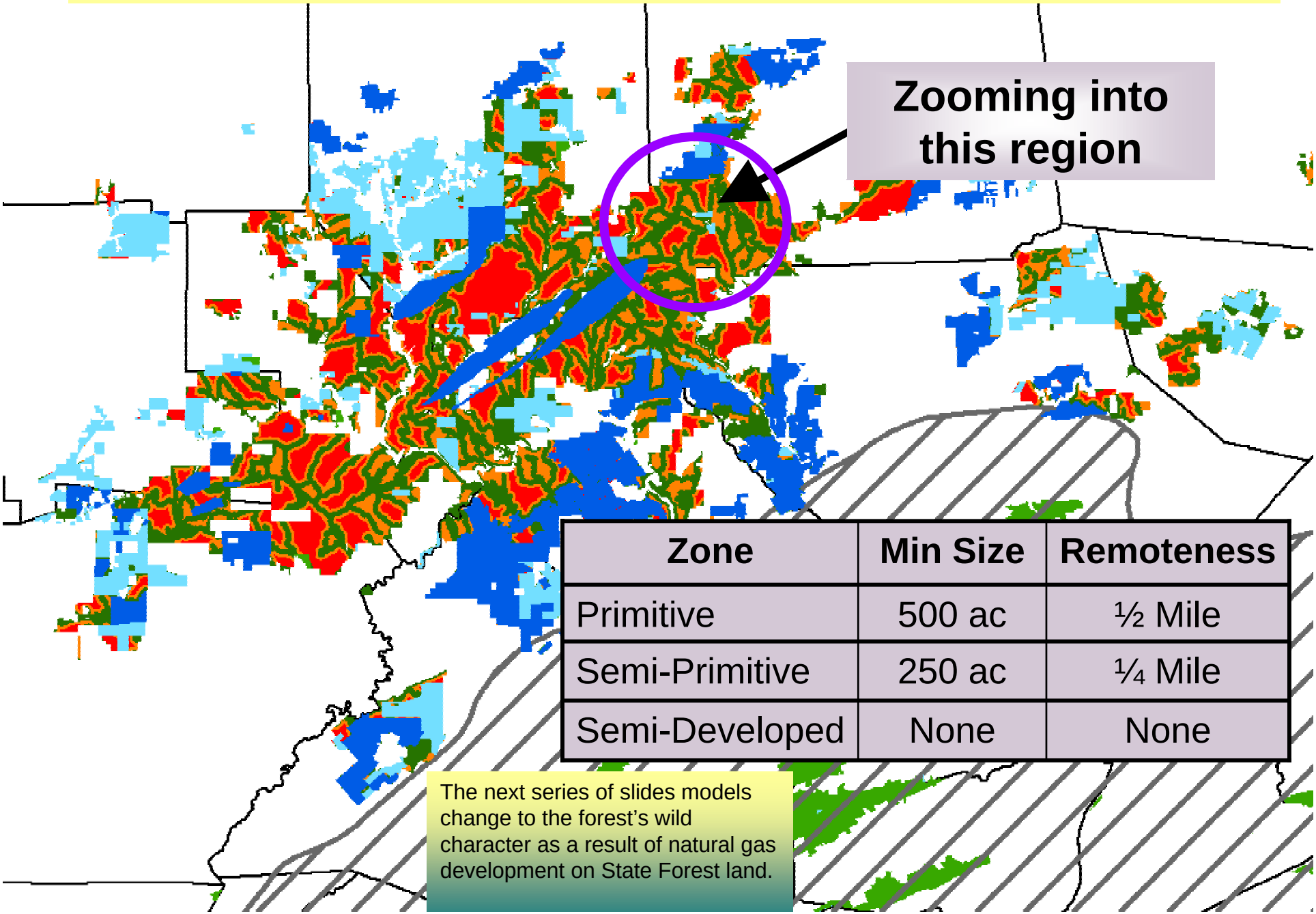

Semi-Primitive areas are shown in **orange**. These areas are moderately remote and contain limited timber and gas activities, but still contribute to the wild characteristics of the forest. Gas development in Semi-Primitive areas can shrink or destroy Primitive areas that are nearby or adjacent.

# Maintaining the Forest's Wild Character




The remaining lands that are not Primitive or Semi-Primitive are shown in **dark green** as Semi-Developed. Encounters with other recreationists, motorized activity, as well as timber and gas activities can be expected here. However, gas development in these areas will shrink or destroy Primitive or Semi-Primitive areas that are nearby or adjacent.

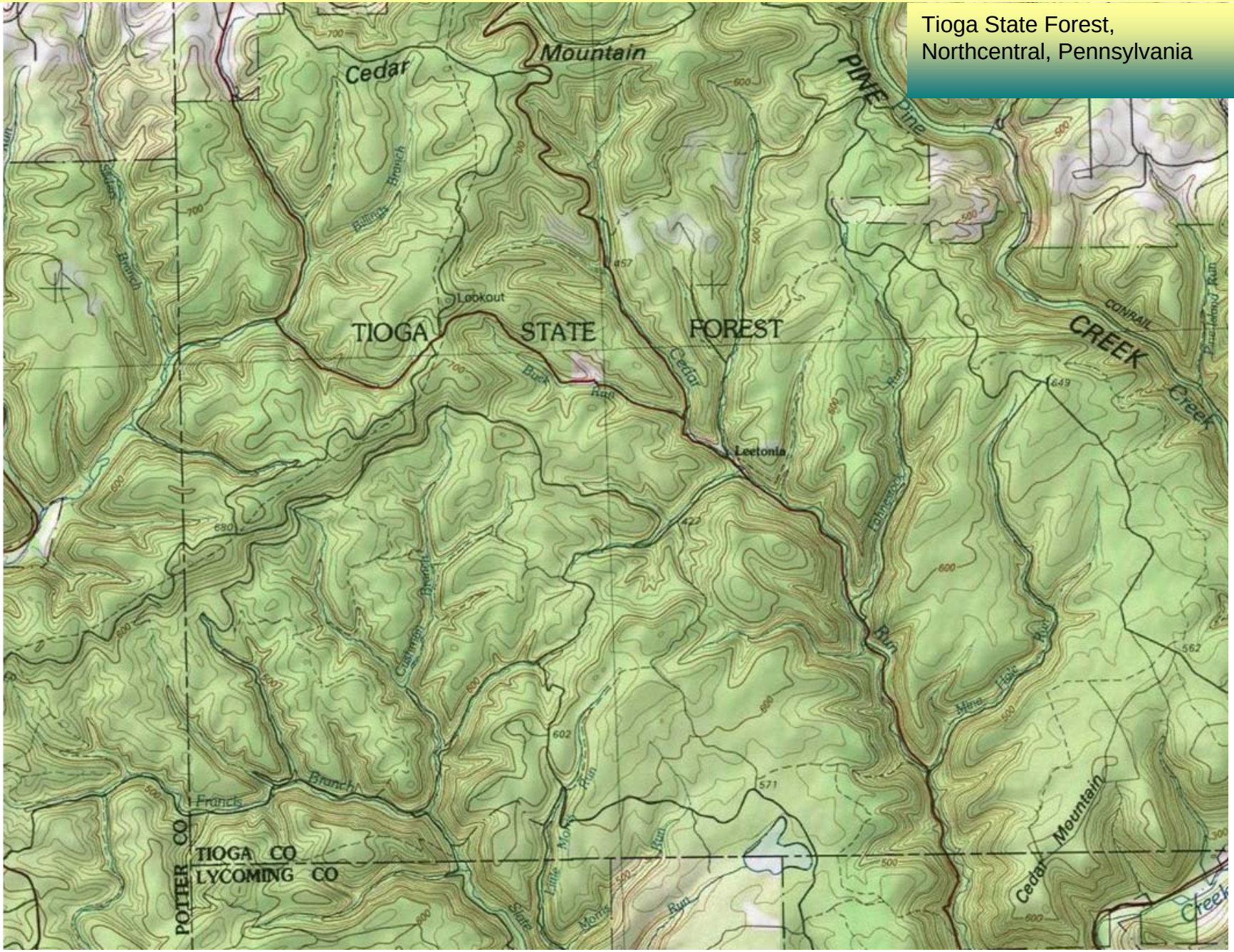
# Impacts on the Wild Character




| Zone | Min Size | Remoteness |
|---|---|---|
| Primitive | 500 ac | ½ Mile |
| Semi-Primitive | 250 ac | ¼ Mile |
| Semi-Developed | None | None |

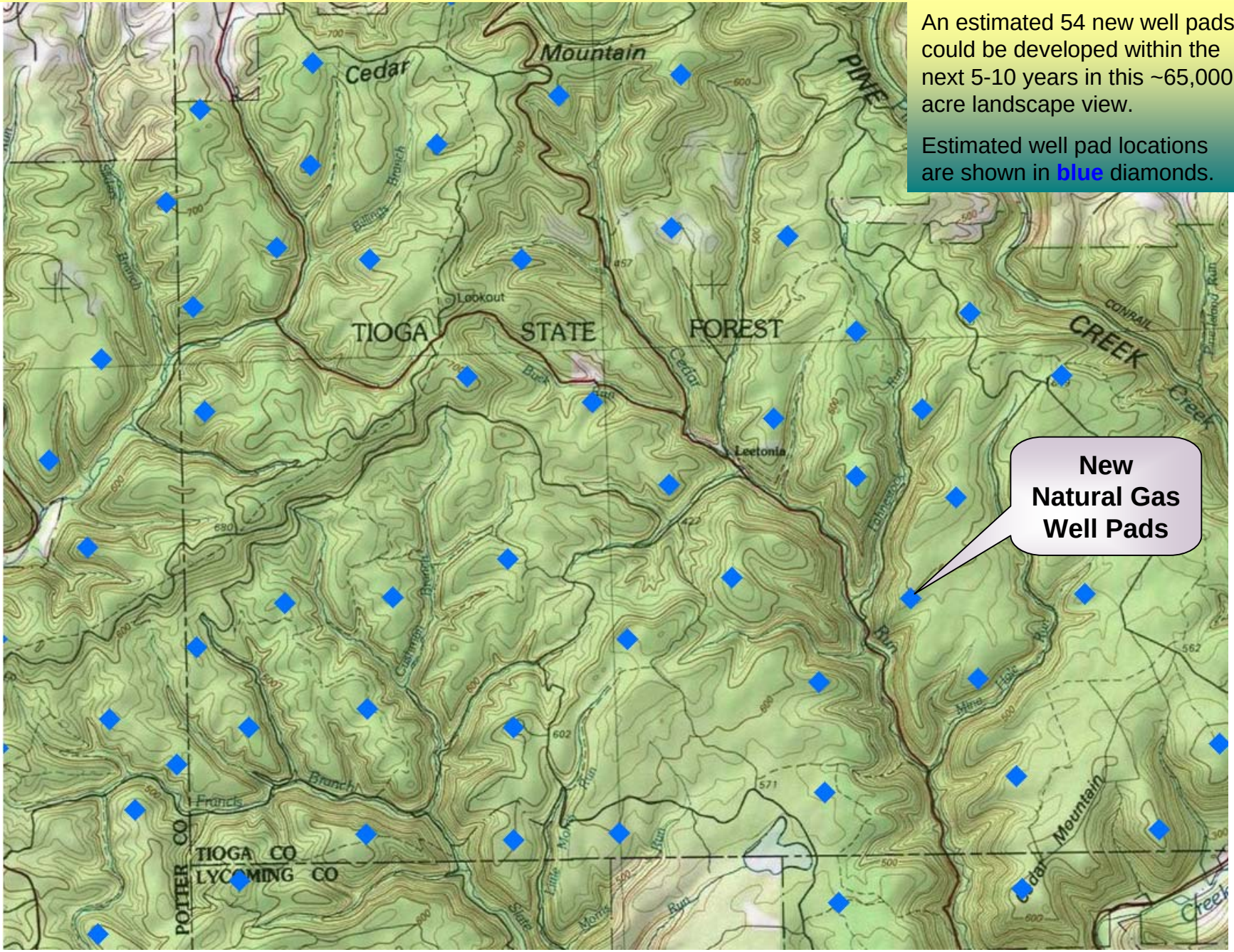
The next series of slides models change to the forest's wild character as a result of natural gas development on State Forest land.

# Topography




Tioga State Forest, Northcentral, Pennsylvania

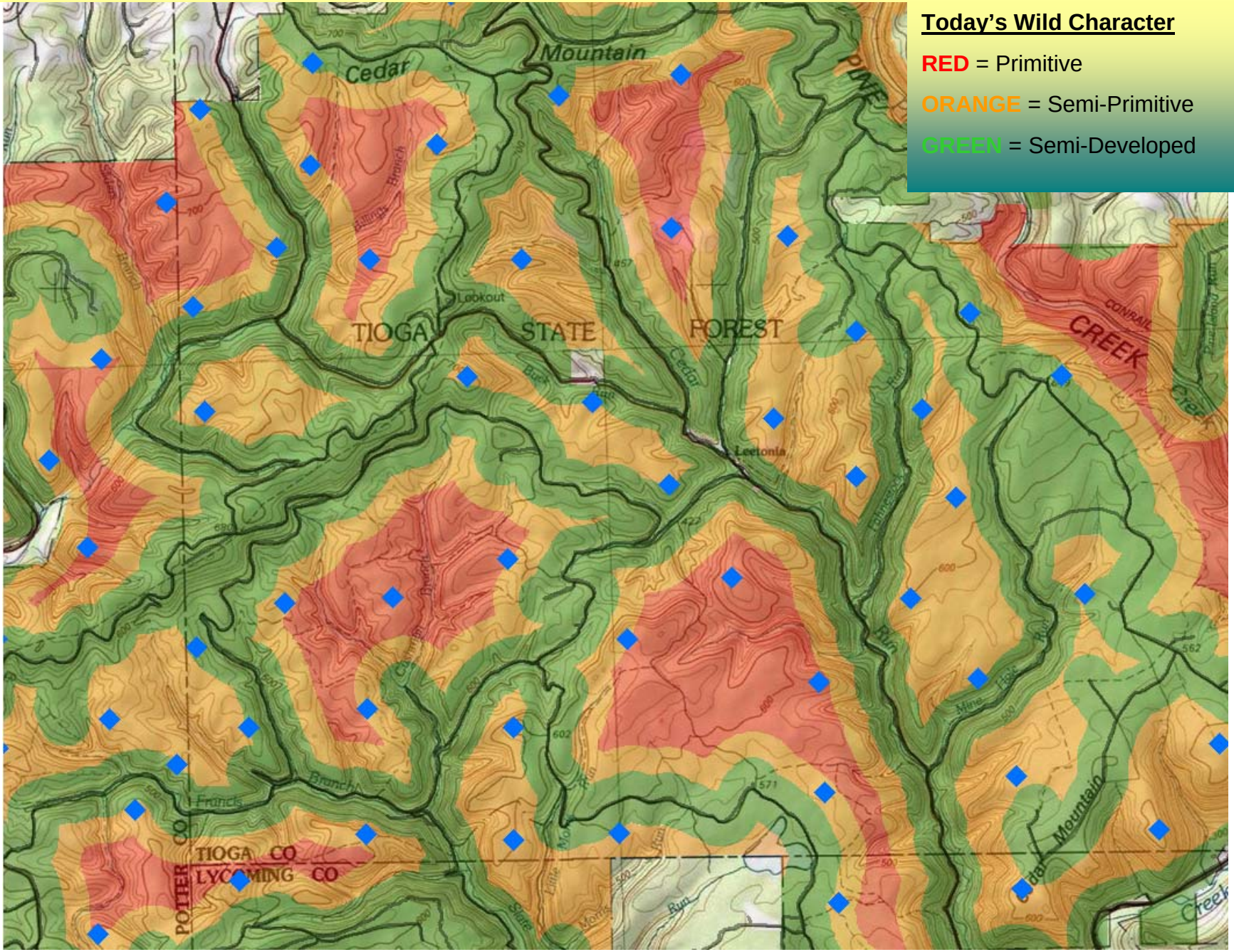
# New Well Pad Locations




An estimated 54 new well pads could be developed within the next 5-10 years in this ~65,000 acre landscape view.

Estimated well pad locations are shown in **blue** diamonds.

# Wild Character before Well Pads



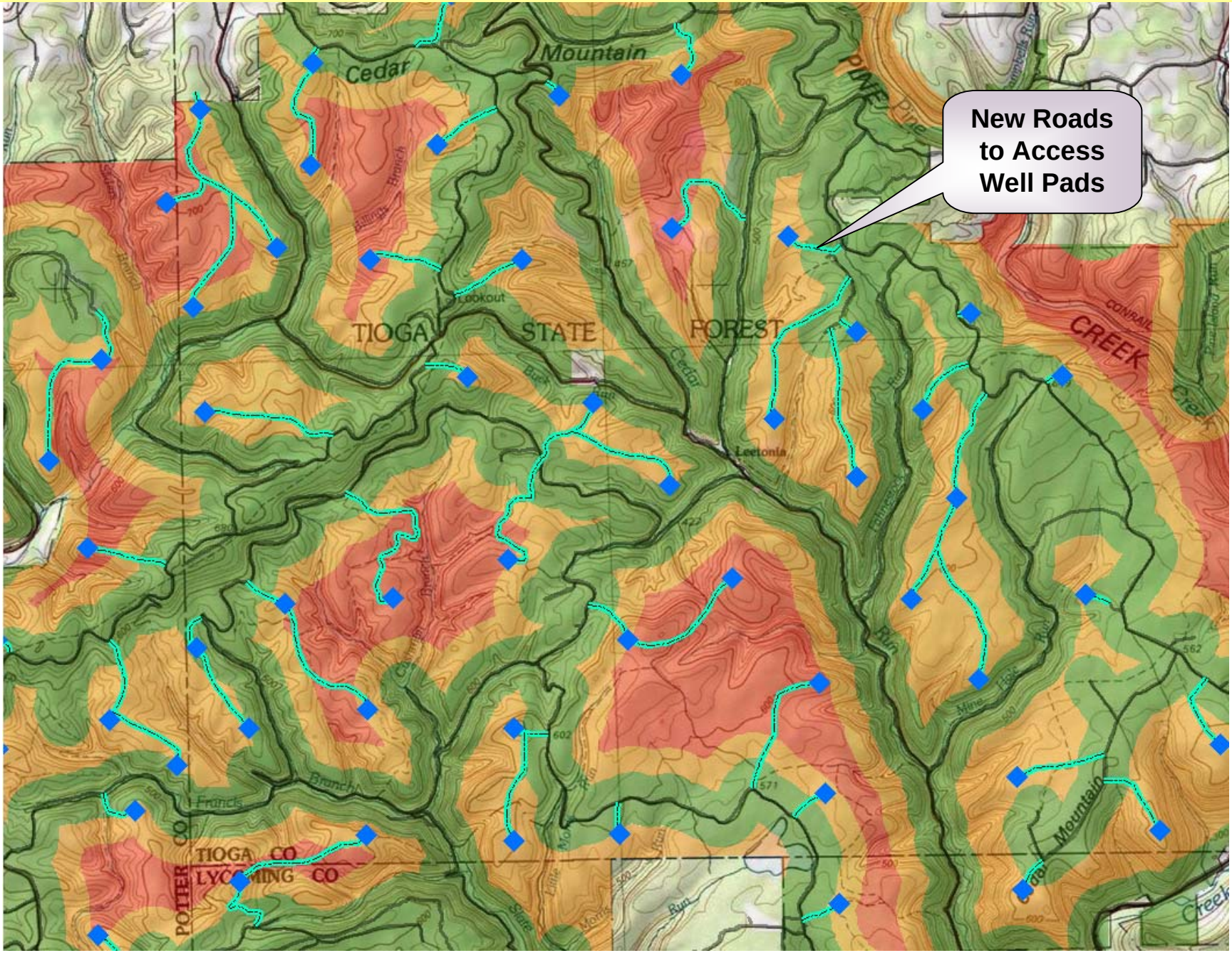

**Today's Wild Character**

RED = Primitive

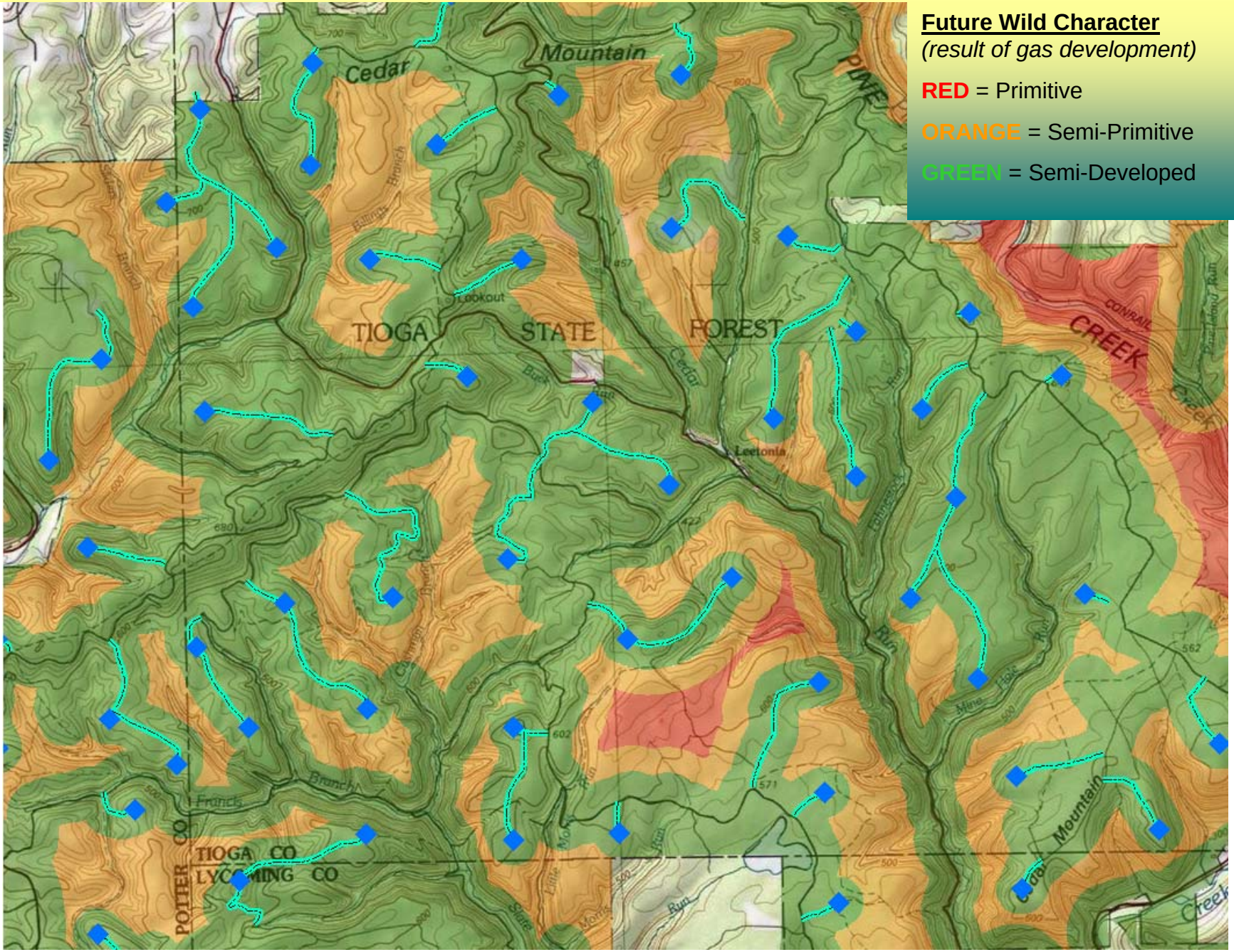
ORANGE = Semi-Primitive

GREEN = Semi-Developed

# New Access Roads Required



# Forest's Wild Character with New Well Pads



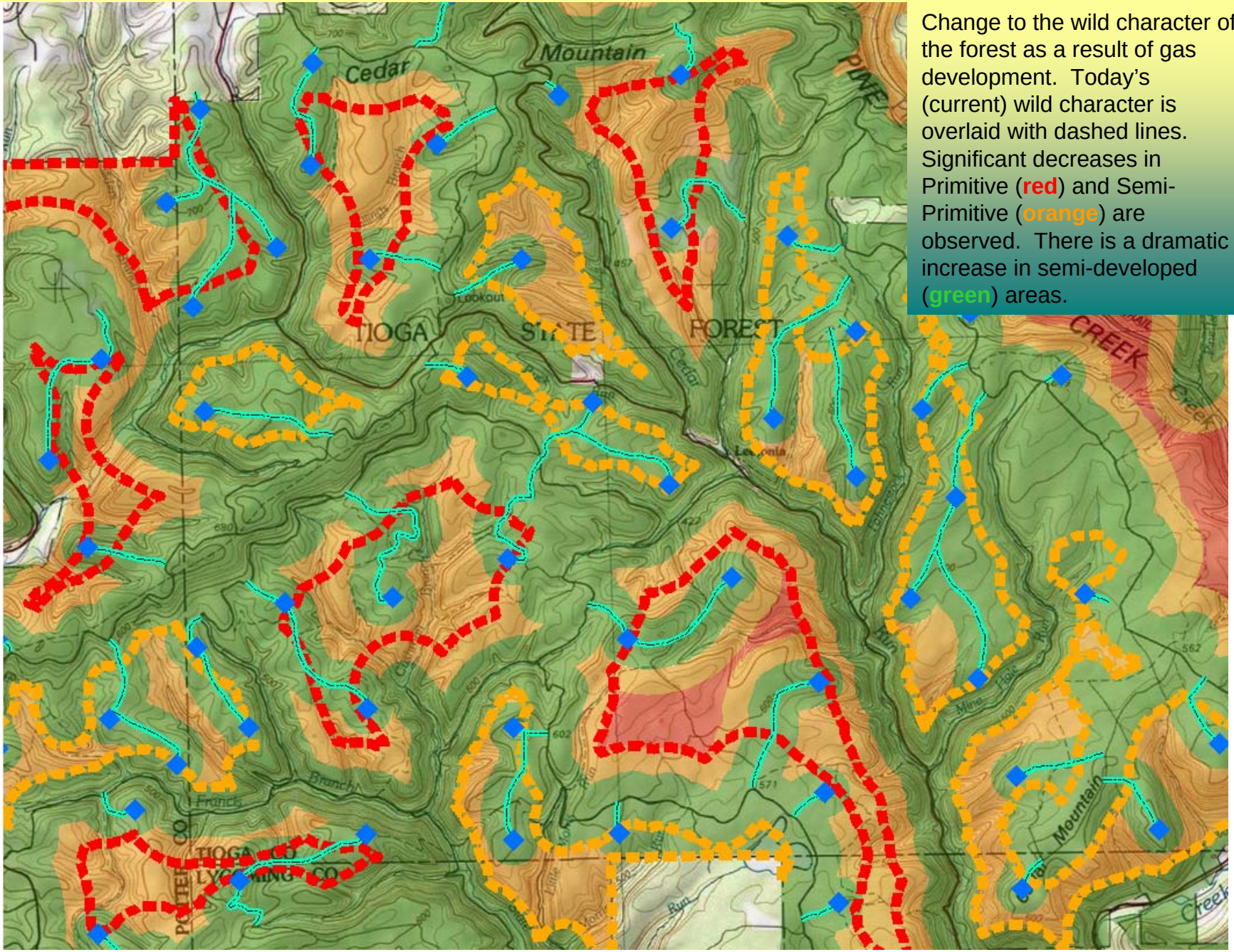

**Future Wild Character**
*(result of gas development)*

**RED** = Primitive

**ORANGE** = Semi-Primitive

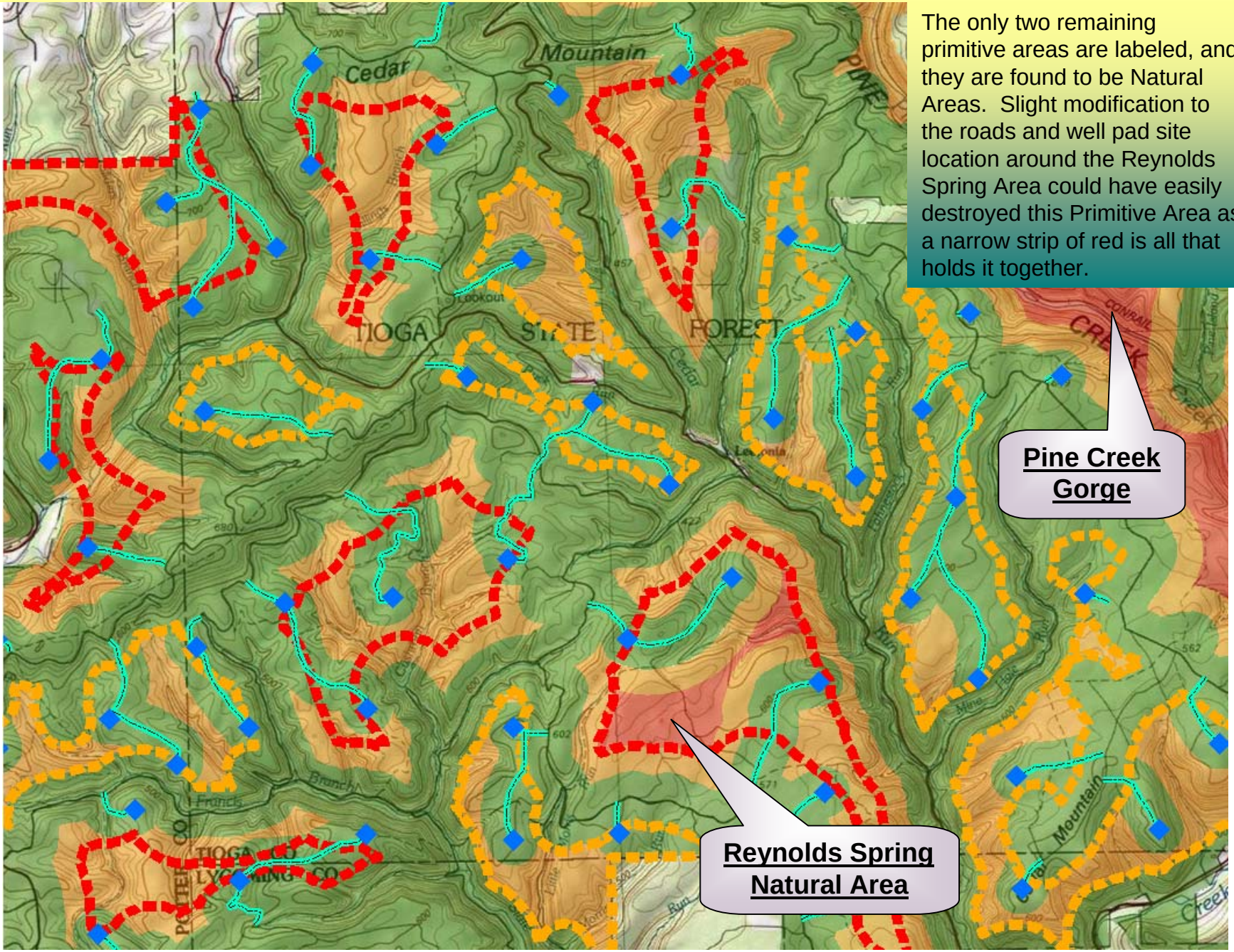
**GREEN** = Semi-Developed

# Impact on the Forest's Wild Character




Change to the wild character of the forest as a result of gas development. Today's (current) wild character is overlaid with dashed lines. Significant decreases in Primitive (**red**) and Semi-Primitive (**orange**) are observed. There is a dramatic increase in semi-developed (**green**) areas.

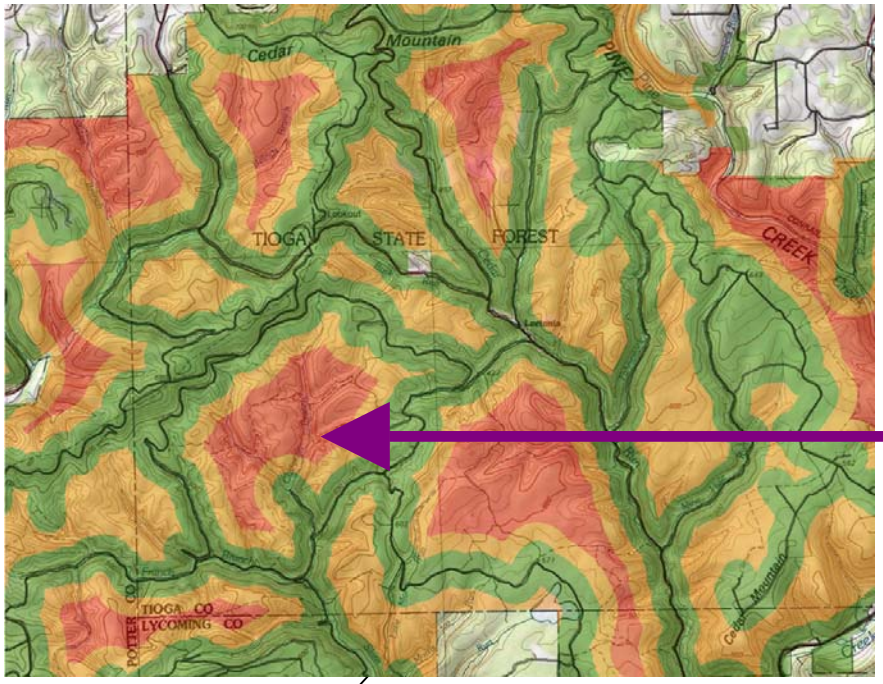
# Impact on the Forest's Wild Character



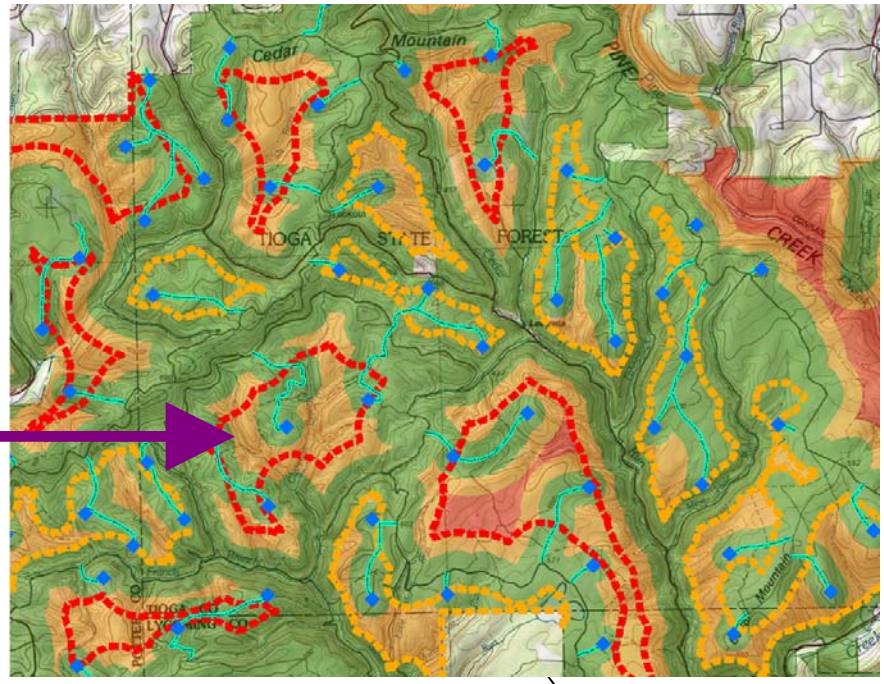

The only two remaining primitive areas are labeled, and they are found to be Natural Areas. Slight modification to the roads and well pad site location around the Reynolds Spring Area could have easily destroyed this Primitive Area as a narrow strip of red is all that holds it together.

# Change in the Forest's Wild Character

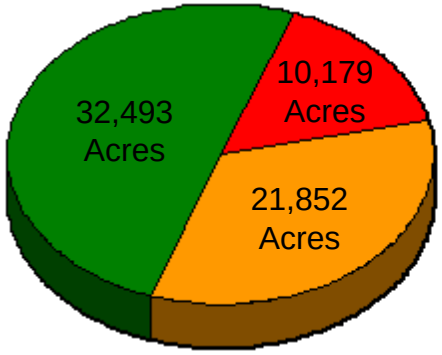
Before



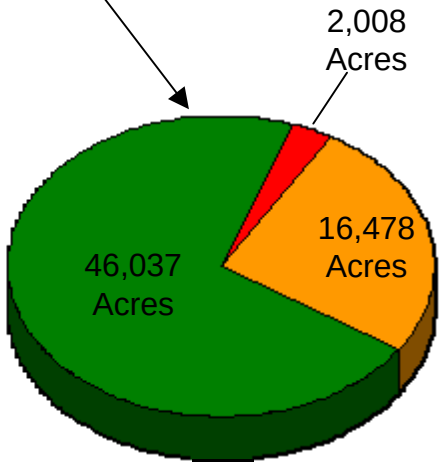

After




In this ~65,000 acre landscape view, with 54 new well pads…



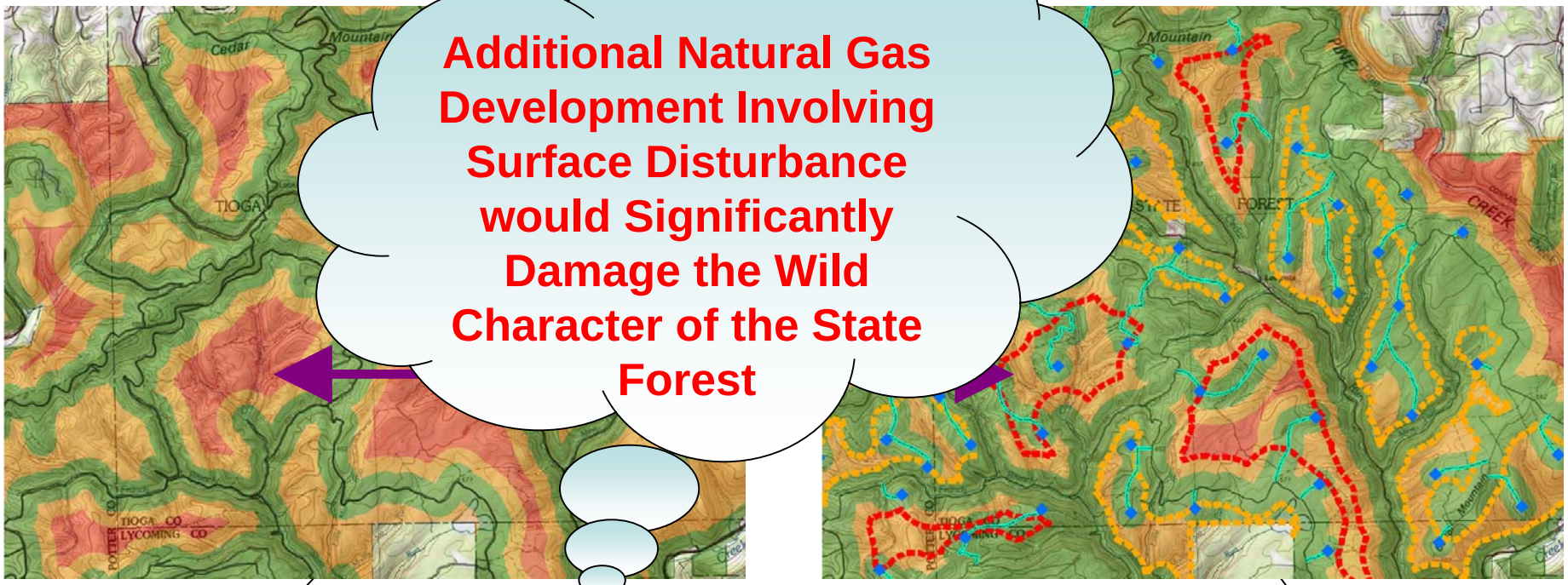

| Zone | Net Gain/Loss |
|---|---|
| Primitive | -8,171 |
| Semi-Primitive | -5,374 |
| Semi-Developed | 13,545 |



# Modeling Change in the Forest's Wild Character

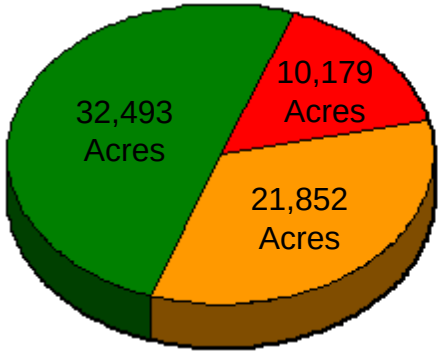
Before

After

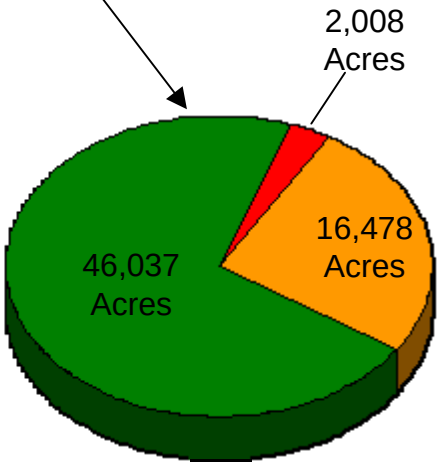



**Additional Natural Gas Development Involving Surface Disturbance would Significantly Damage the Wild Character of the State Forest**

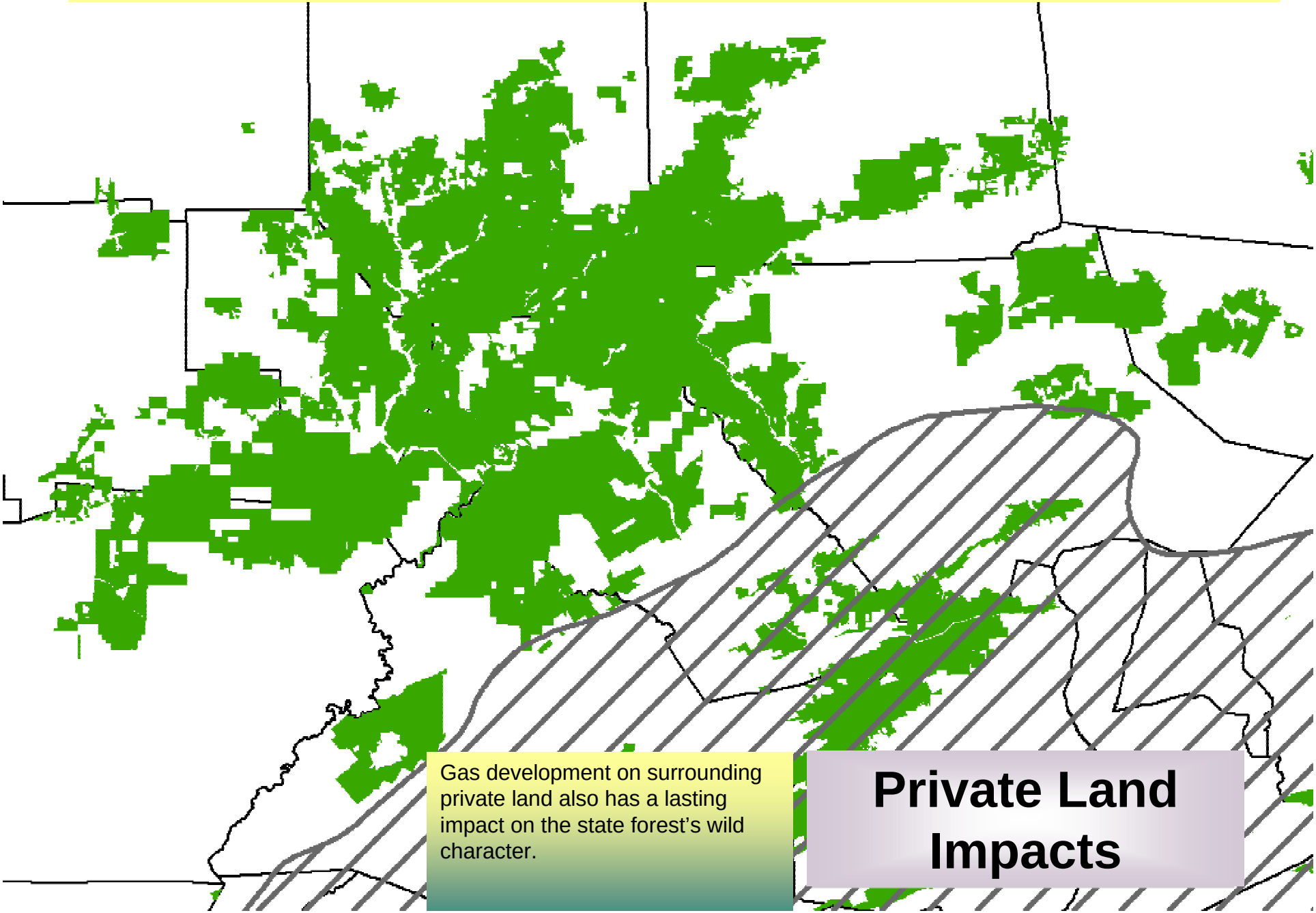
In this ~65,000 acre landscape view, with 54 new well pads…




| Zone | Net Gain/Loss |
|---|---|
| Primitive | -8,171 |
| Semi-Primitive | -5,374 |
| Semi-Developed | 13,545 |

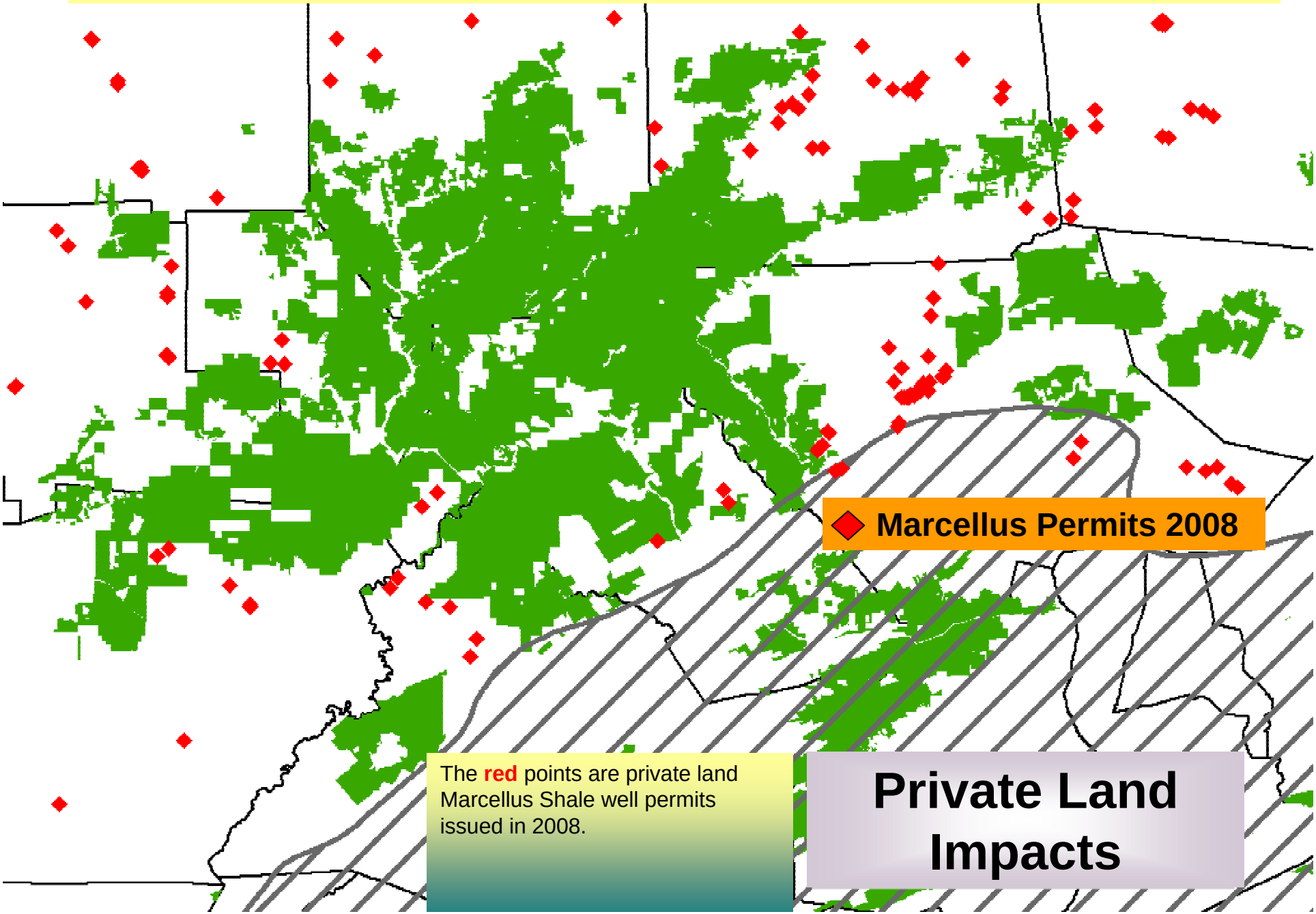


# Maintaining the Forest's Wild Character




Gas development on surrounding private land also has a lasting impact on the state forest's wild character.

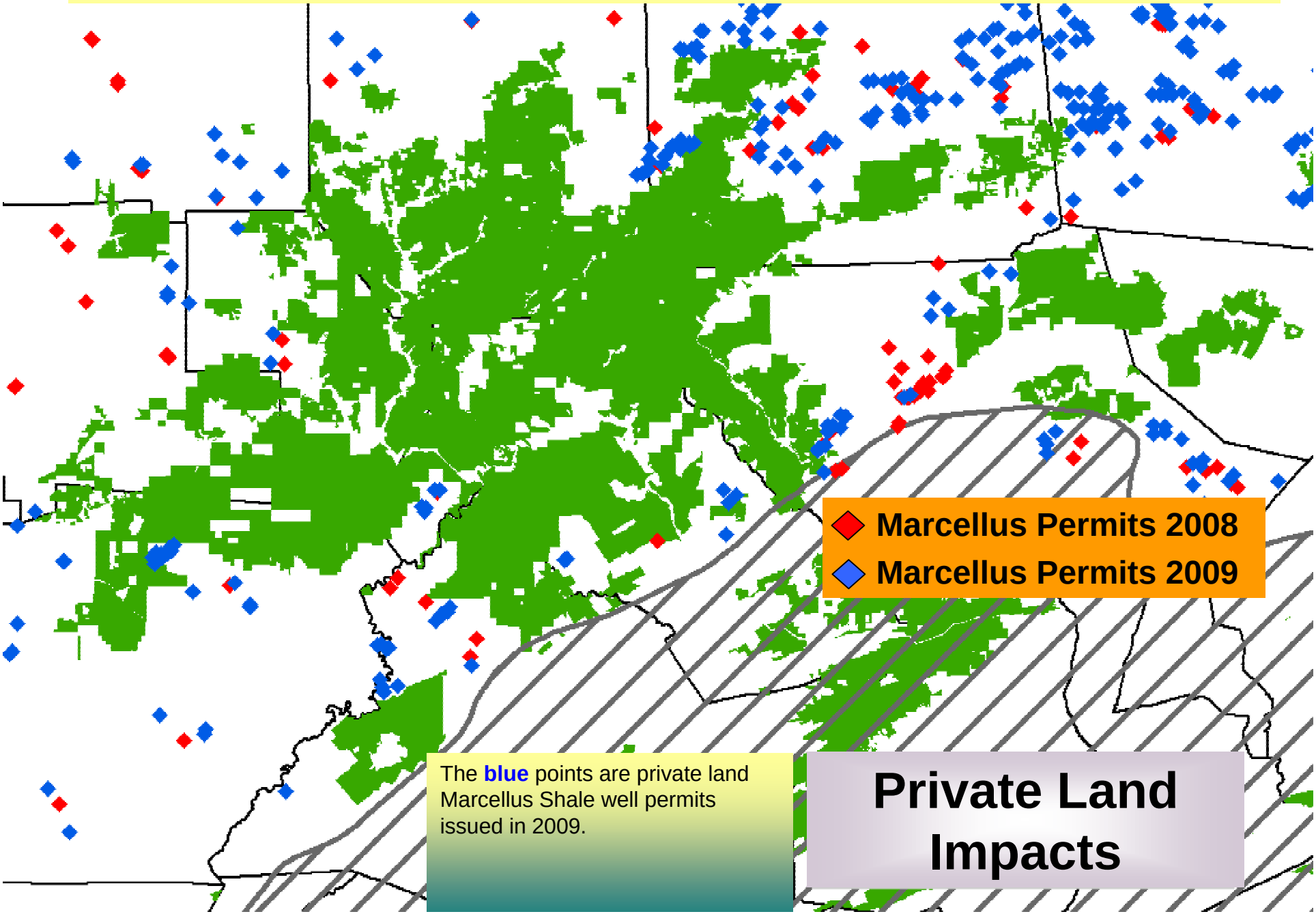
# Maintaining the Forest's Wild Character




The **red** points are private land Marcellus Shale well permits issued in 2008.

**Private Land Impacts**

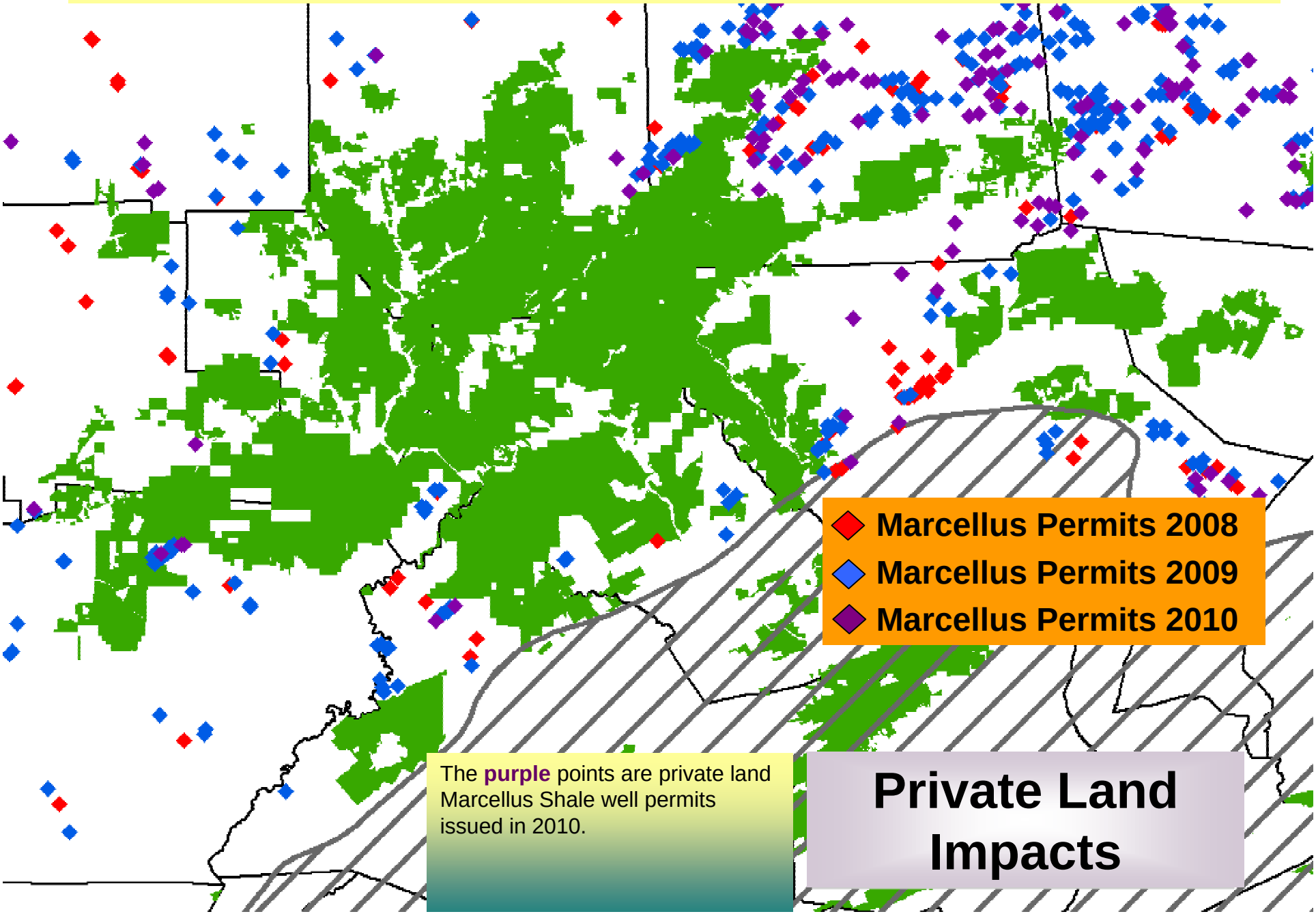
# Maintaining the Forest's Wild Character




The **blue** points are private land Marcellus Shale well permits issued in 2009.

**Private Land Impacts**

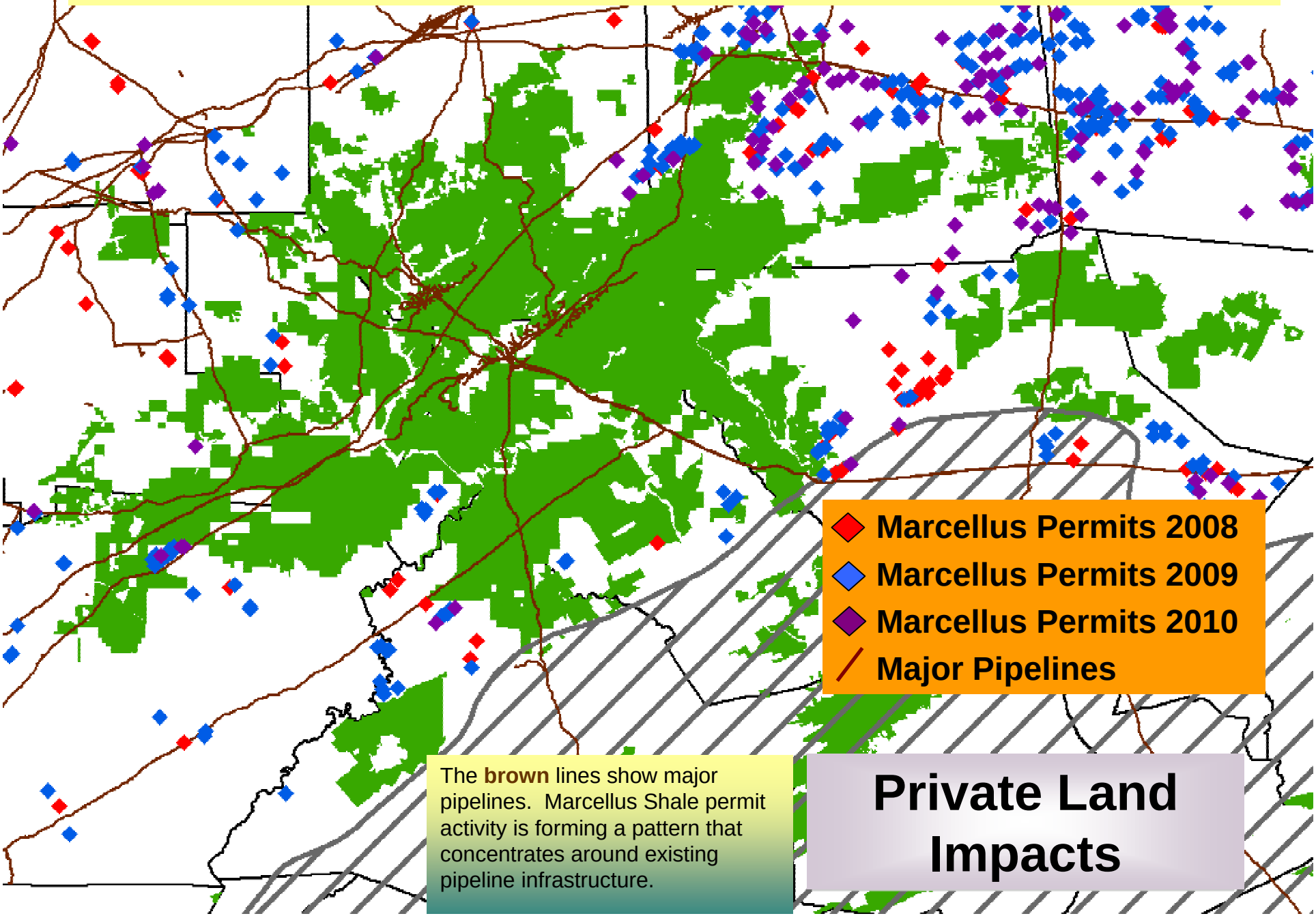
# Maintaining the Forest's Wild Character




The **purple** points are private land Marcellus Shale well permits issued in 2010.

**Private Land Impacts**

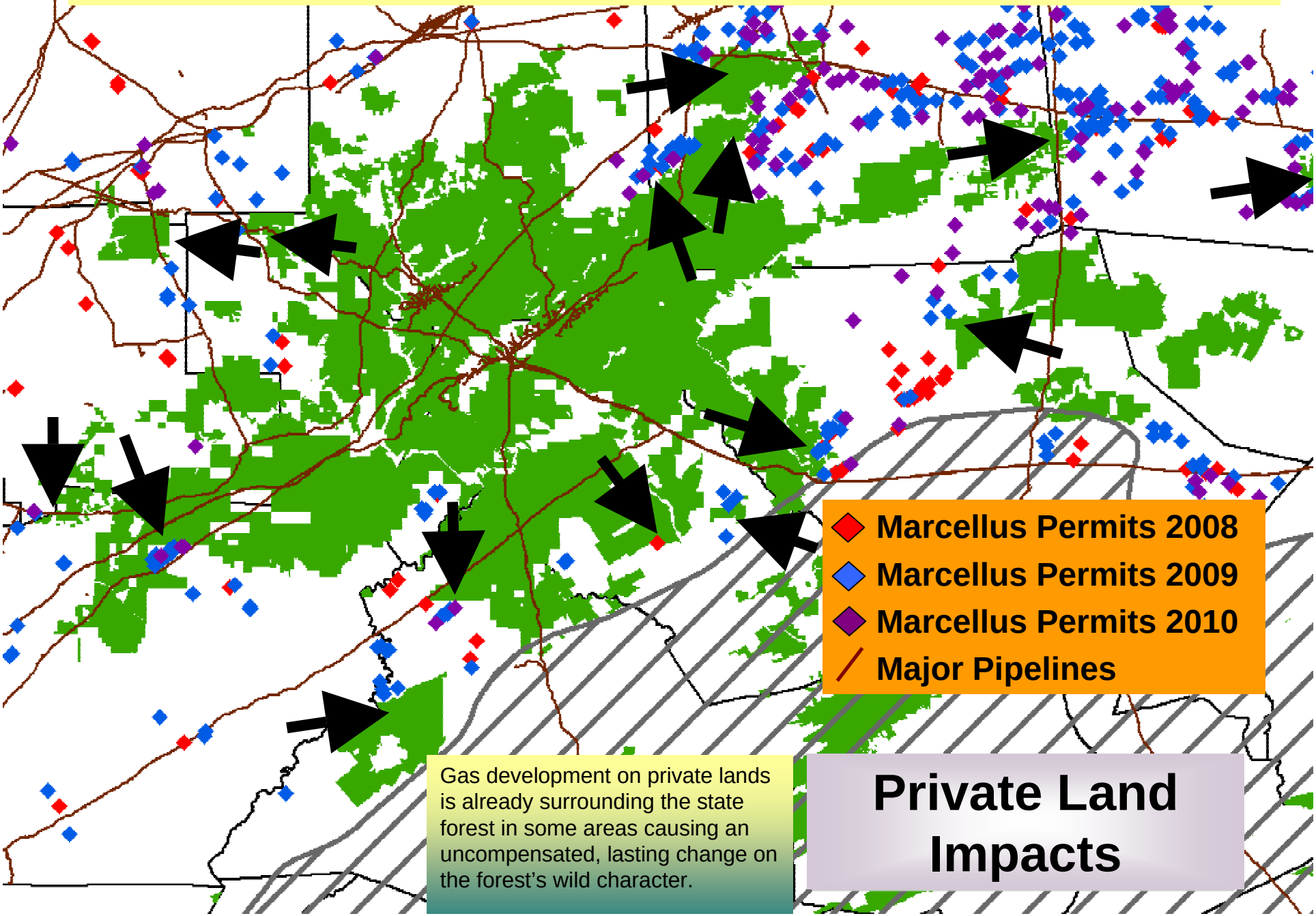
# Maintaining the Forest's Wild Character




The **brown** lines show major pipelines. Marcellus Shale permit activity is forming a pattern that concentrates around existing pipeline infrastructure.

**Private Land Impacts**

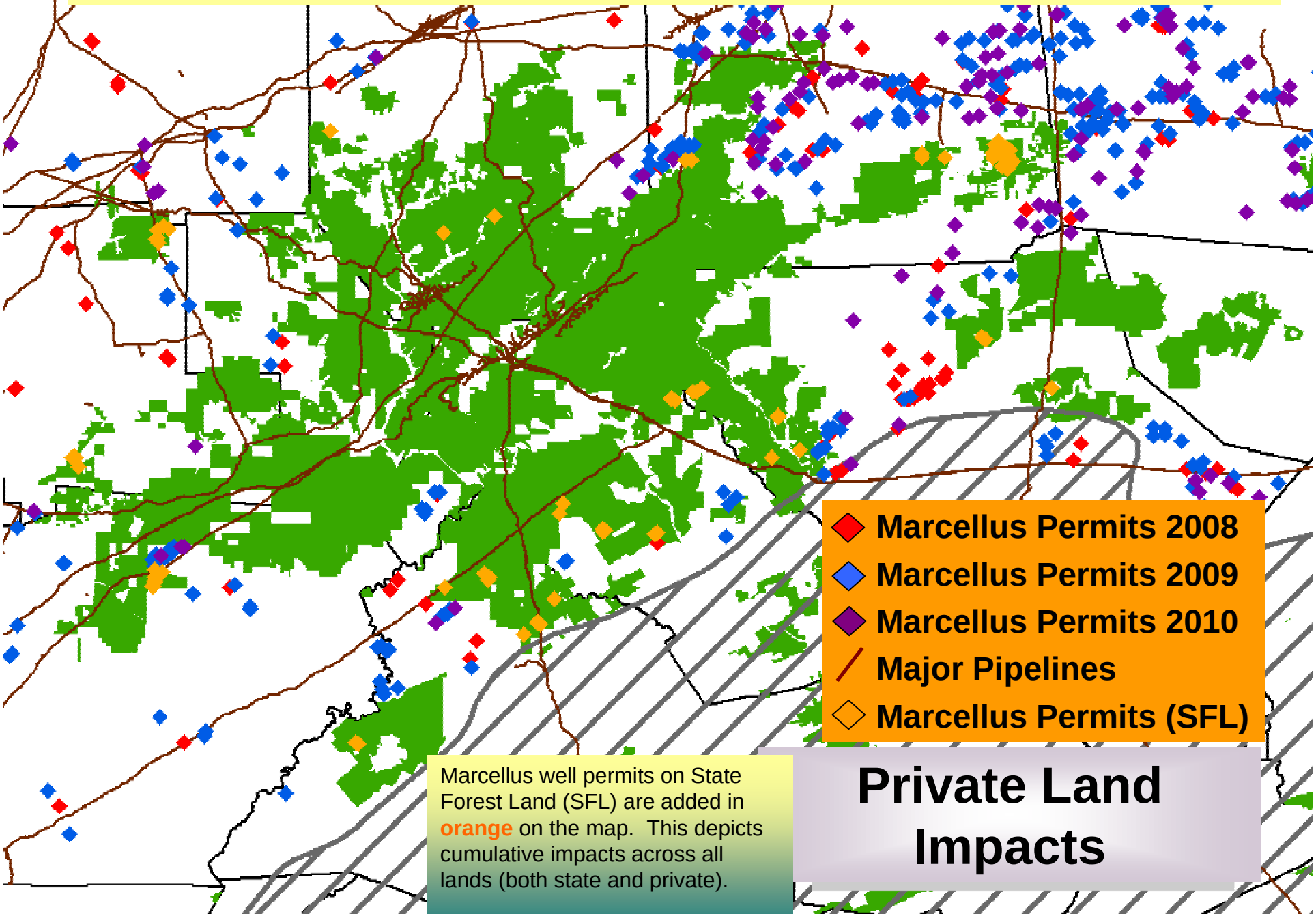
# Maintaining the Forest's Wild Character




Gas development on private lands is already surrounding the state forest in some areas causing an uncompensated, lasting change on the forest's wild character.

**Private Land Impacts**

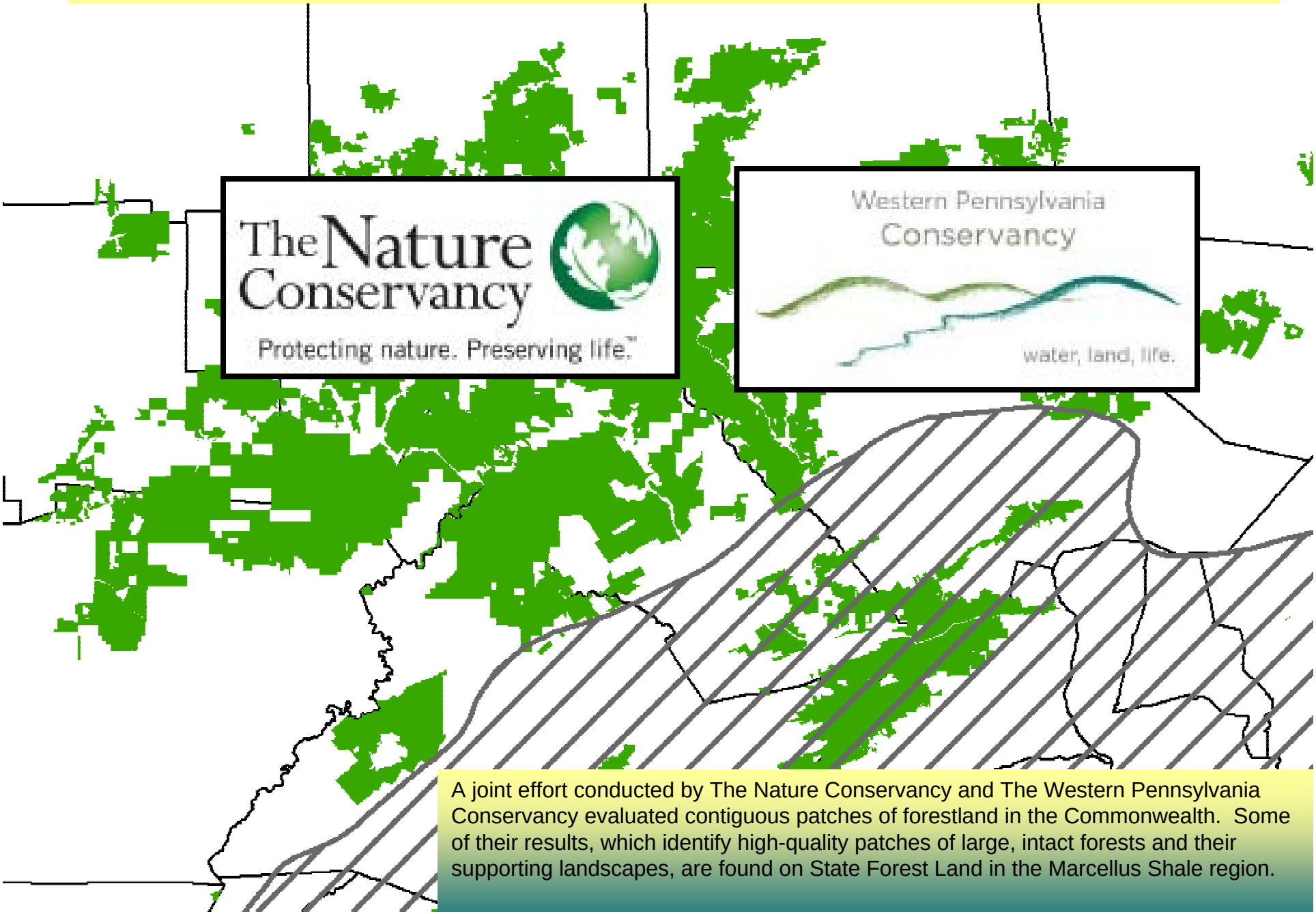
# Maintaining the Forest's Wild Character




Marcellus well permits on State Forest Land (SFL) are added in **orange** on the map. This depicts cumulative impacts across all lands (both state and private).

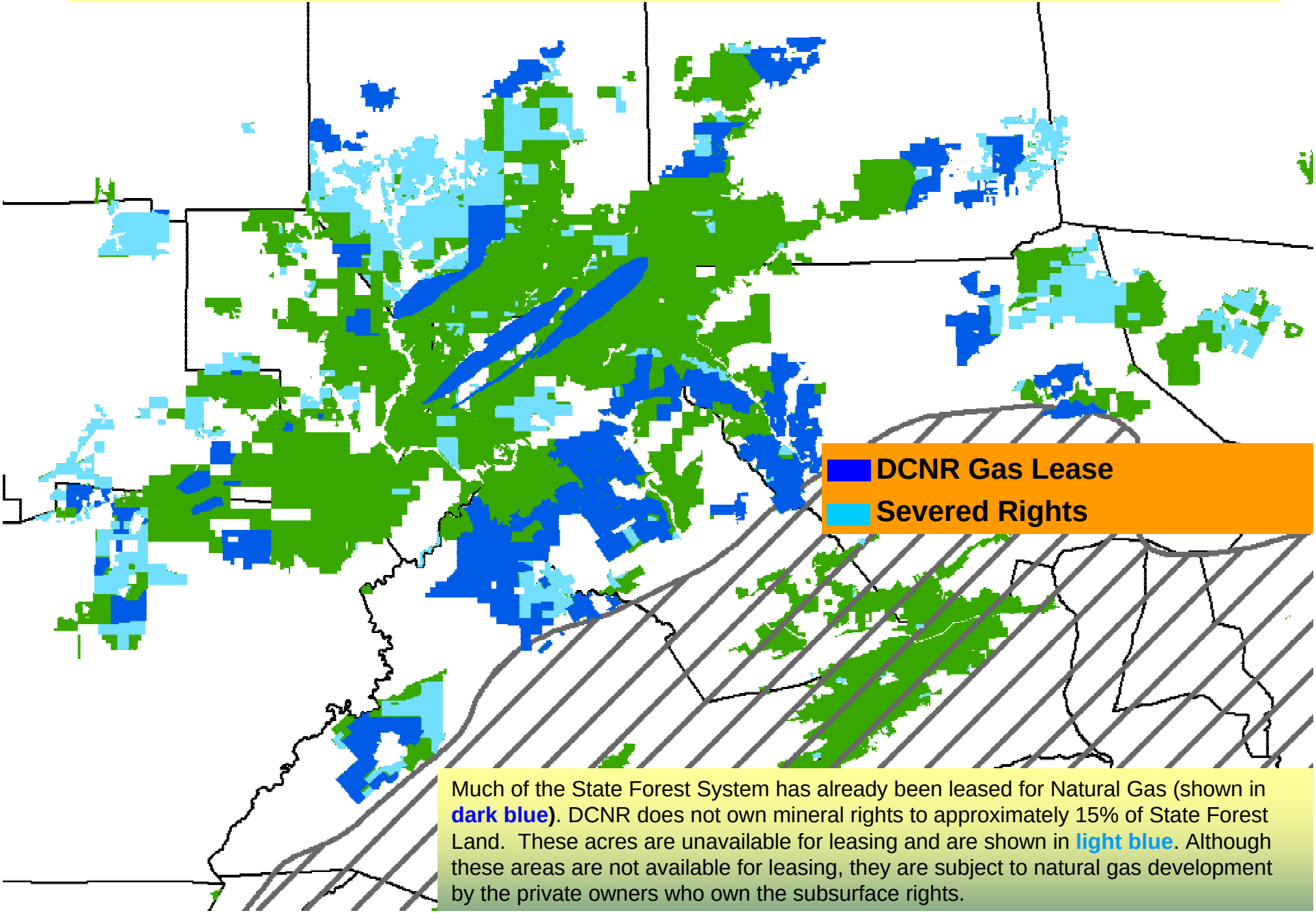
## Private Land Impacts

# TNC-WPC Priority Forest Patches



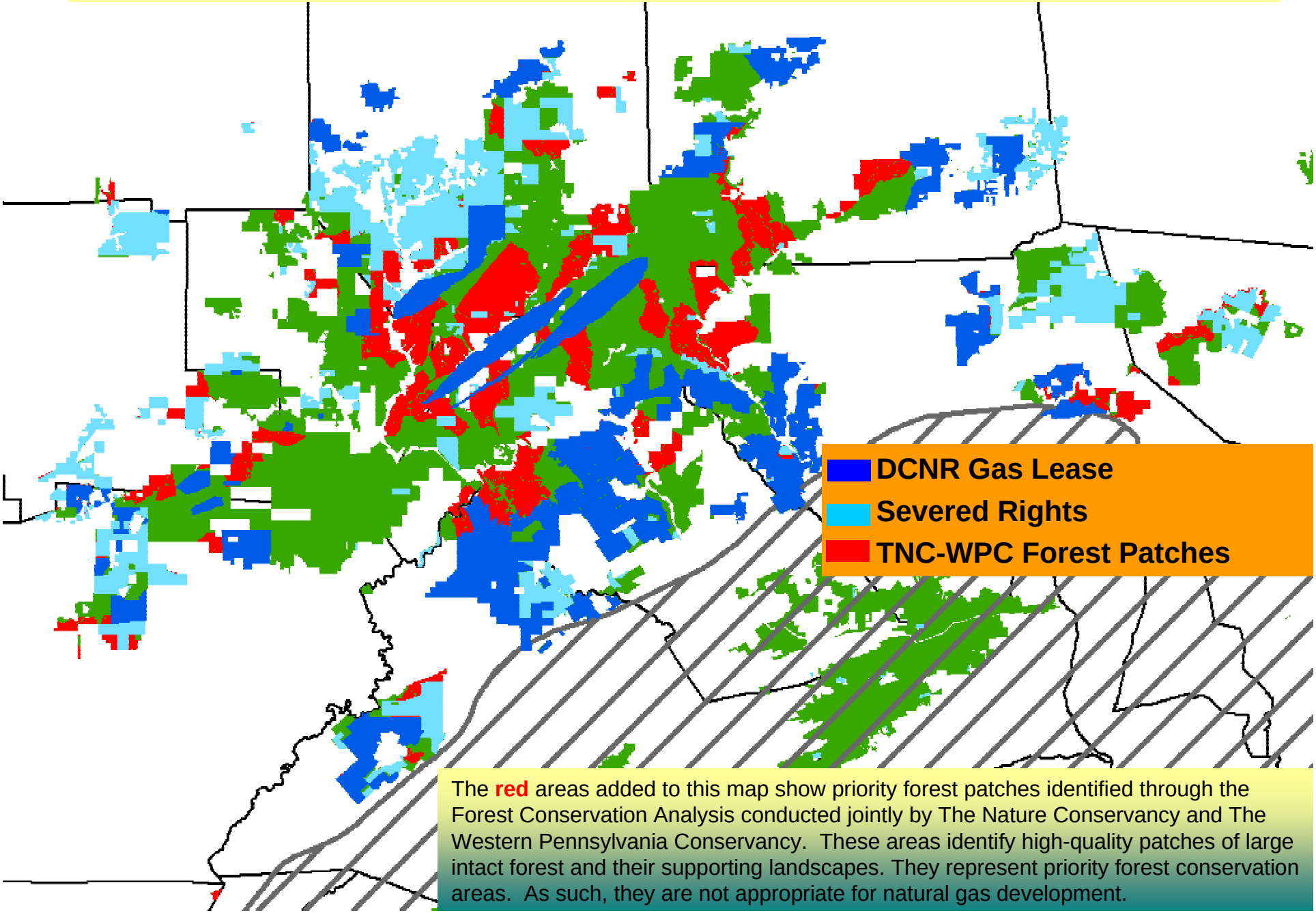

A joint effort conducted by The Nature Conservancy and The Western Pennsylvania Conservancy evaluated contiguous patches of forestland in the Commonwealth. Some of their results, which identify high-quality patches of large, intact forests and their supporting landscapes, are found on State Forest Land in the Marcellus Shale region.

# TNC-WPC Priority Forest Patches



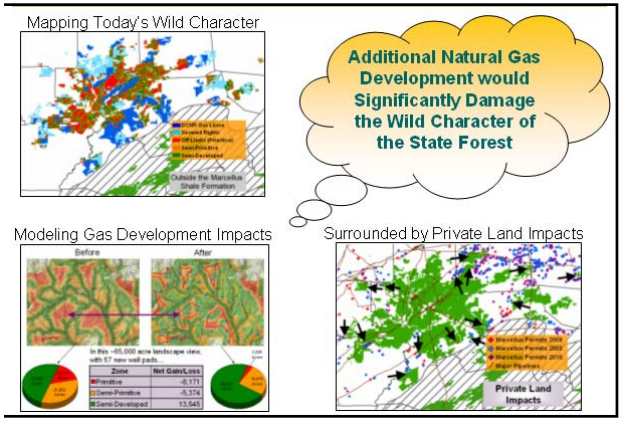

Much of the State Forest System has already been leased for Natural Gas (shown in **dark blue**). DCNR does not own mineral rights to approximately 15% of State Forest Land. These acres are unavailable for leasing and are shown in **light blue**. Although these areas are not available for leasing, they are subject to natural gas development by the private owners who own the subsurface rights.

# TNC-WPC Priority Forest Patches



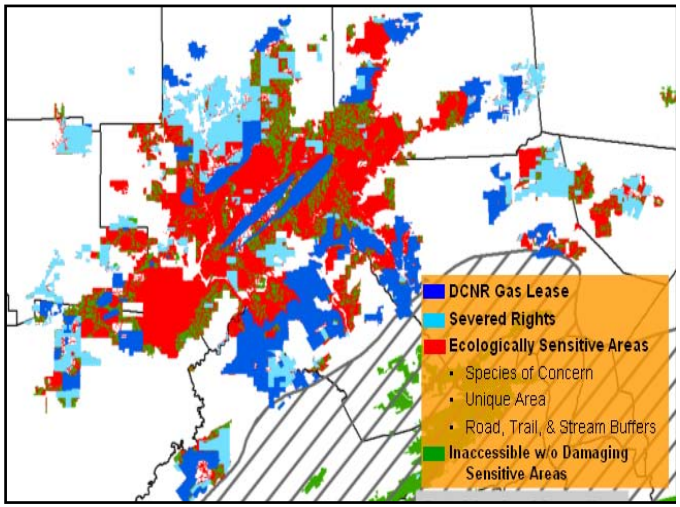

The **red** areas added to this map show priority forest patches identified through the Forest Conservation Analysis conducted jointly by The Nature Conservancy and The Western Pennsylvania Conservancy. These areas identify high-quality patches of large intact forest and their supporting landscapes. They represent priority forest conservation areas. As such, they are not appropriate for natural gas development.

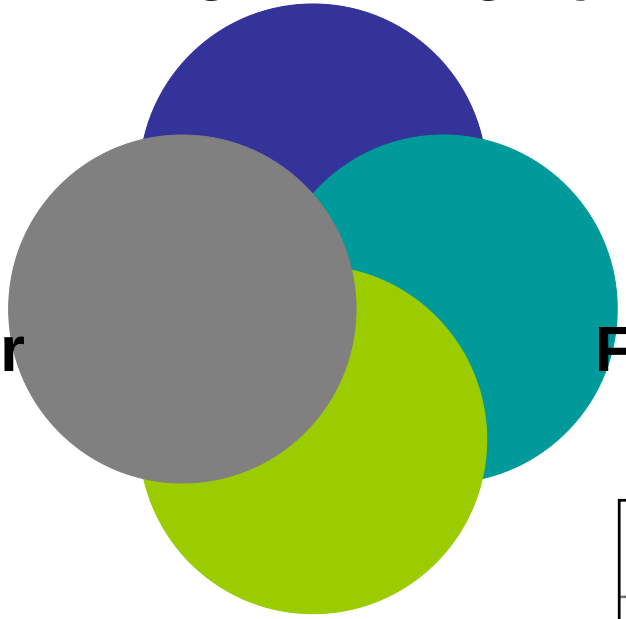
# Cumulative Assessment & Impacts



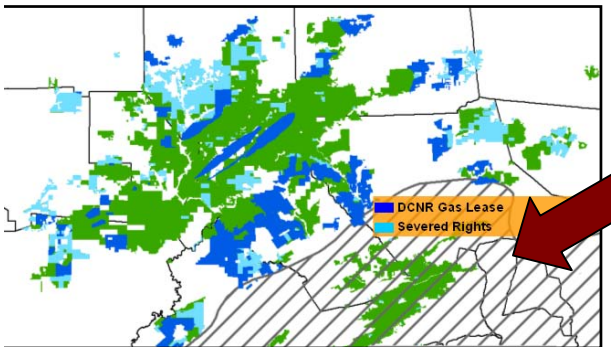

**The Forest's Wild Character**

**The Forest's Ecological Integrity**

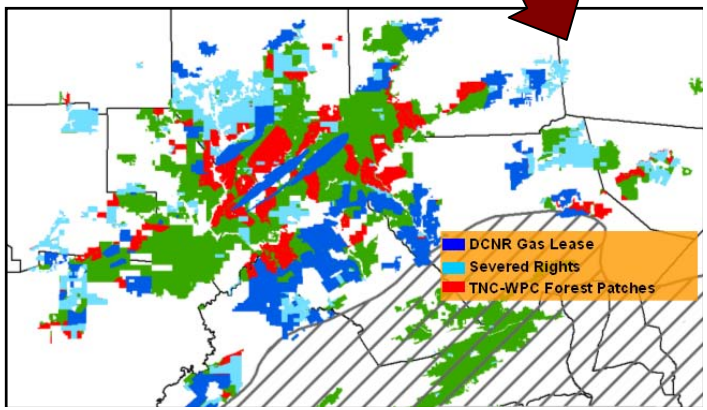


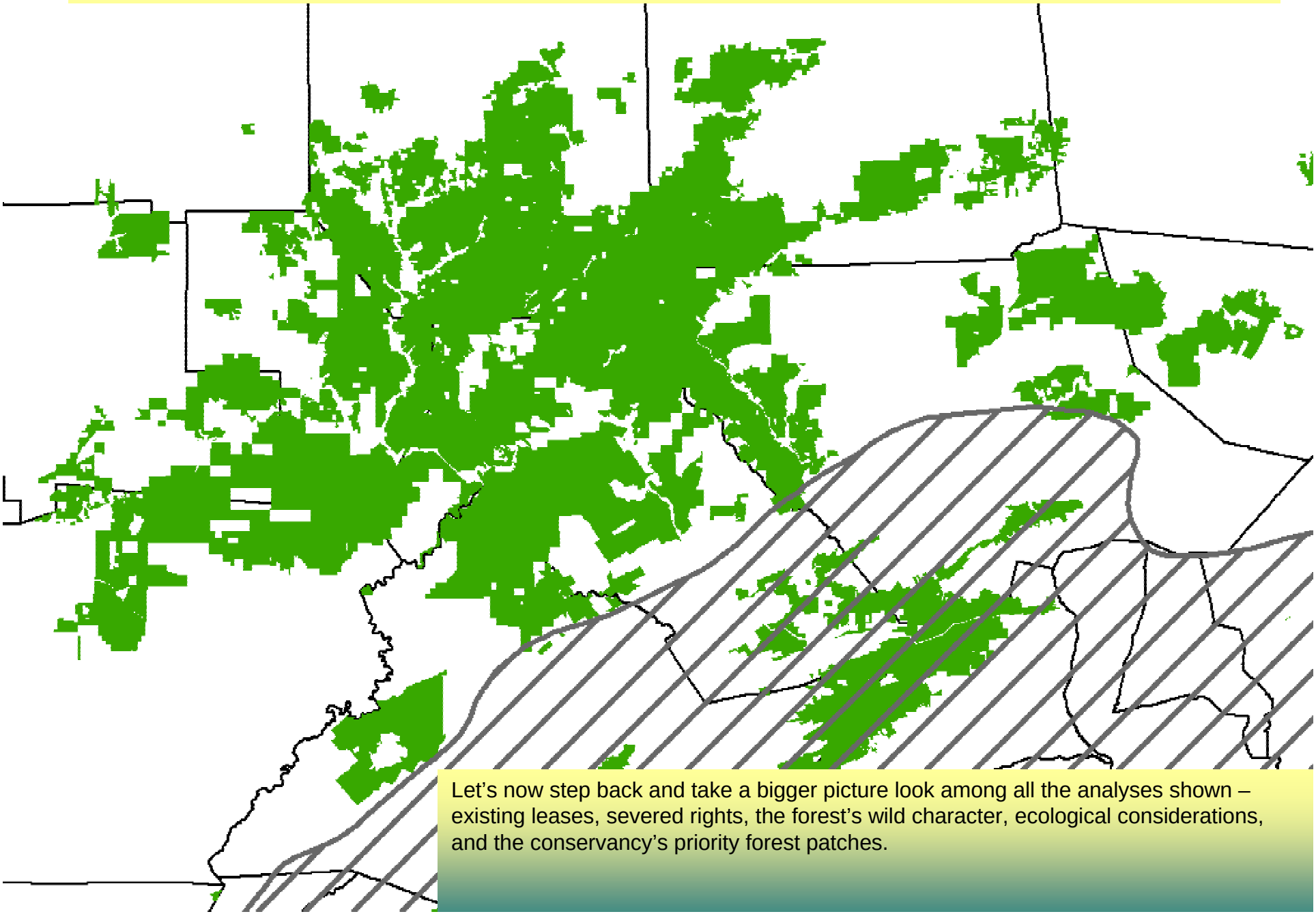
**TNC-WPC Forest Patches**




**Existing Leases & Severed Rights**

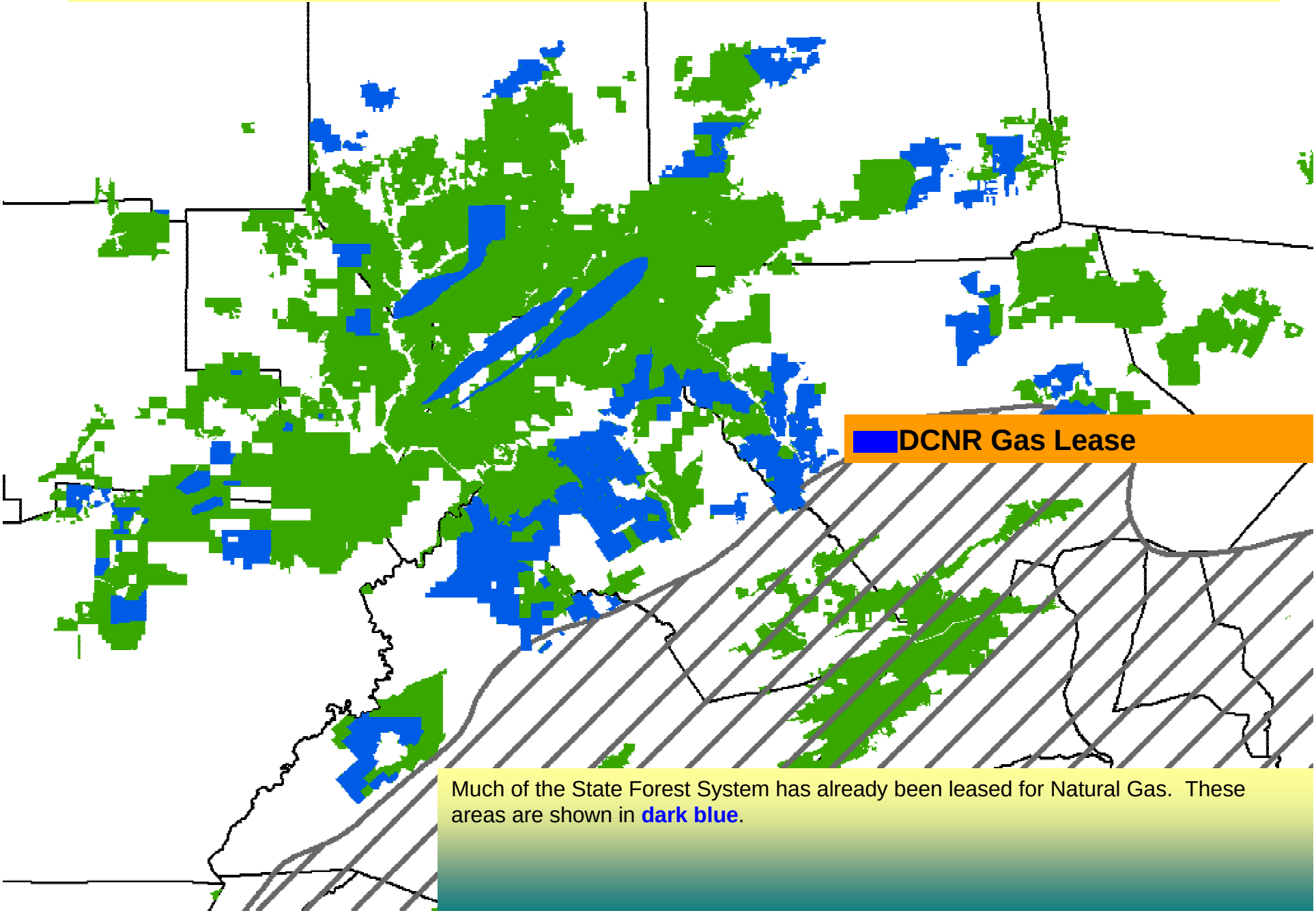


# Cumulative Assessment & Impacts



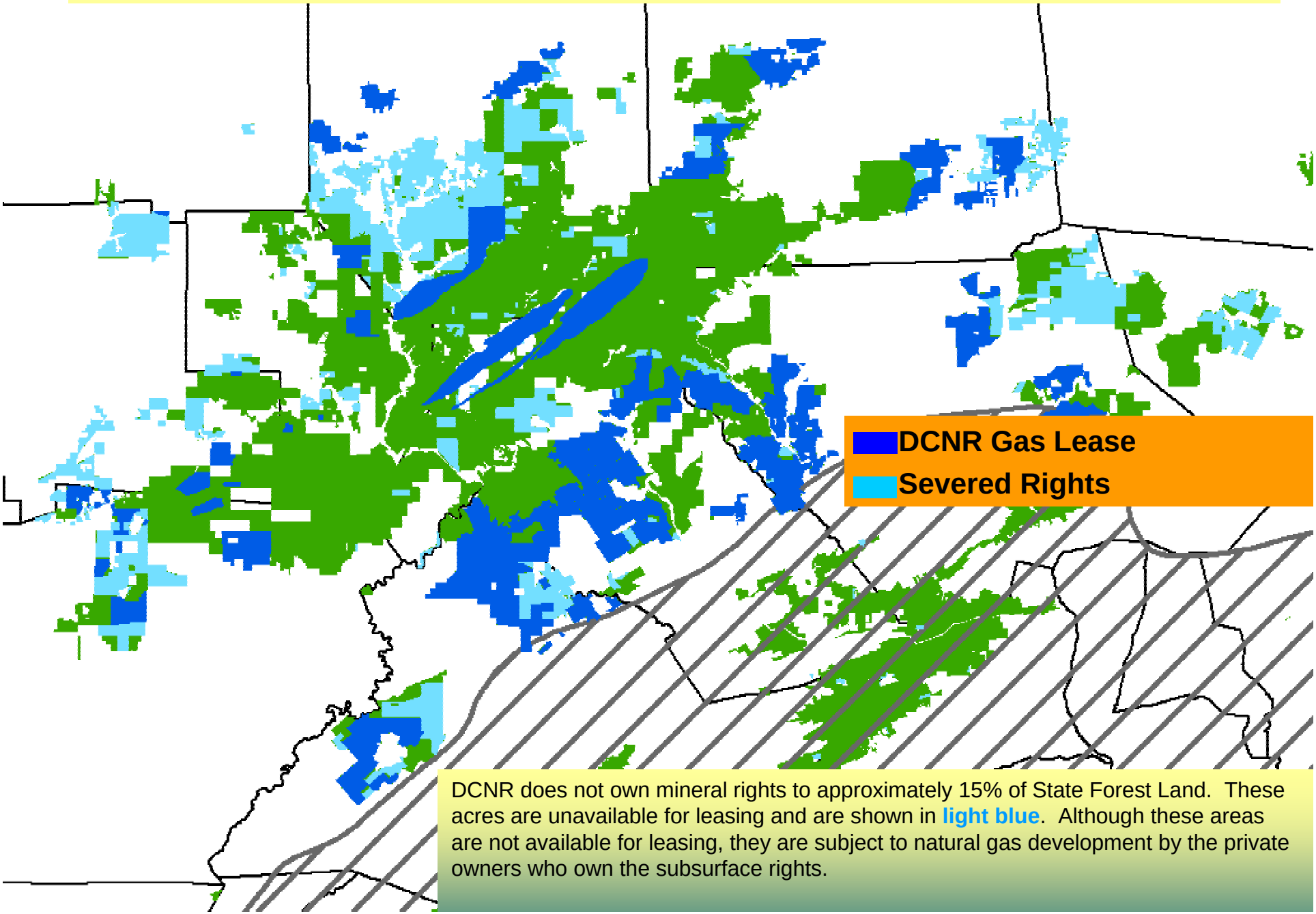
Let's now step back and take a bigger picture look among all the analyses shown – existing leases, severed rights, the forest's wild character, ecological considerations, and the conservancy's priority forest patches.

# Cumulative Assessment & Impacts



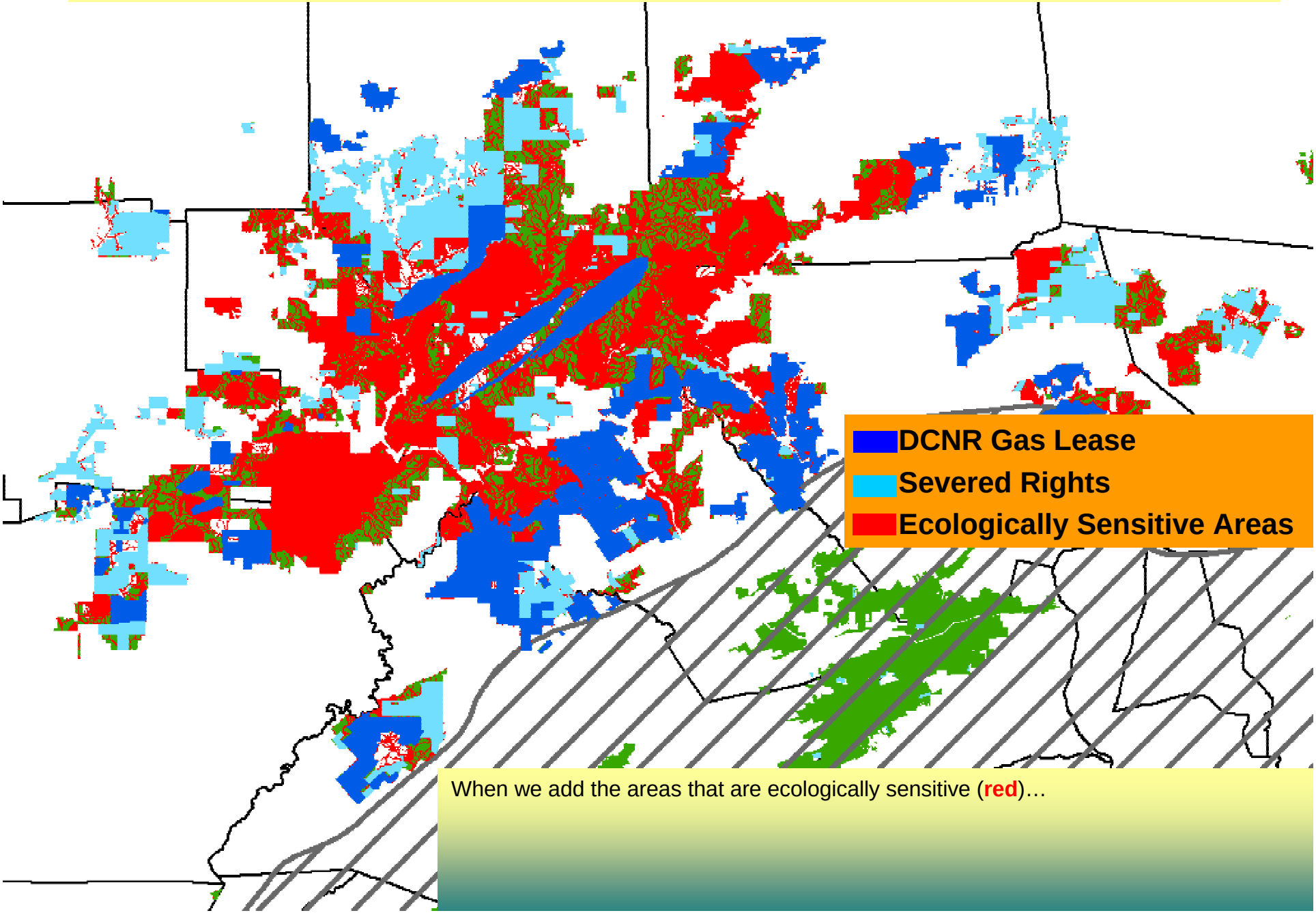

Much of the State Forest System has already been leased for Natural Gas. These areas are shown in **dark blue**.

# Cumulative Assessment & Impacts



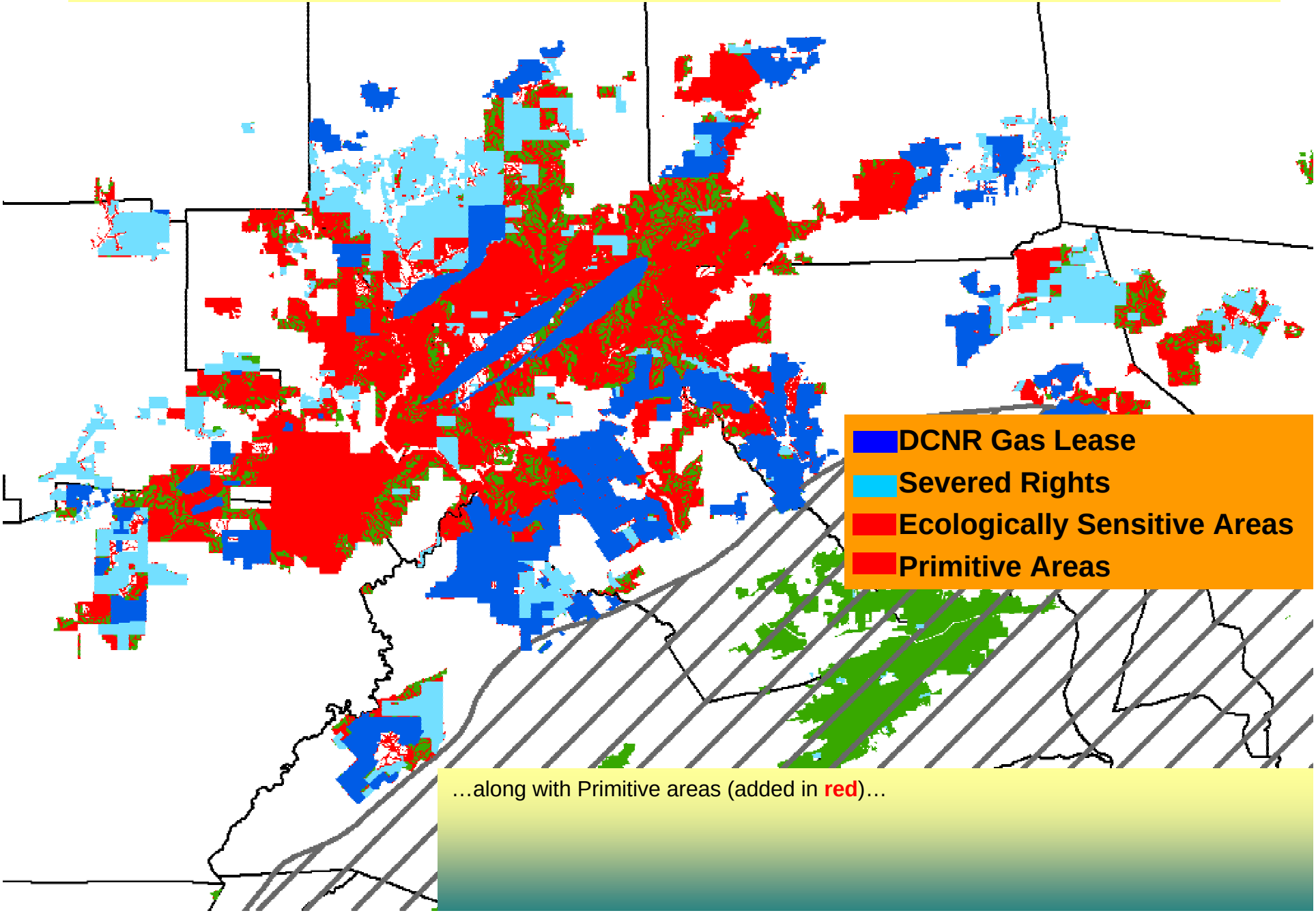

DCNR does not own mineral rights to approximately 15% of State Forest Land. These acres are unavailable for leasing and are shown in **light blue**. Although these areas are not available for leasing, they are subject to natural gas development by the private owners who own the subsurface rights.

# Cumulative Assessment & Impacts



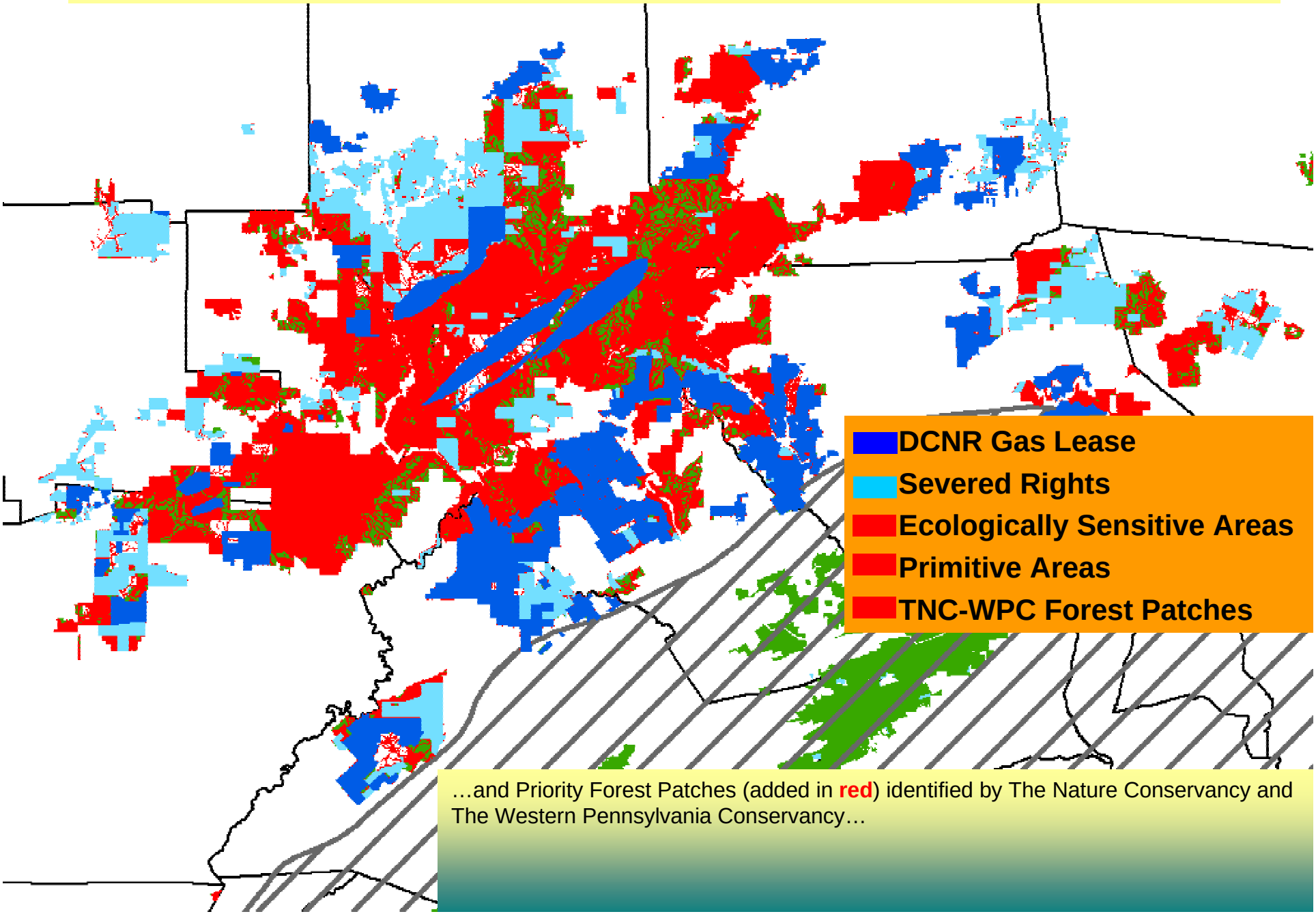

When we add the areas that are ecologically sensitive (**red**)…

# Cumulative Assessment & Impacts



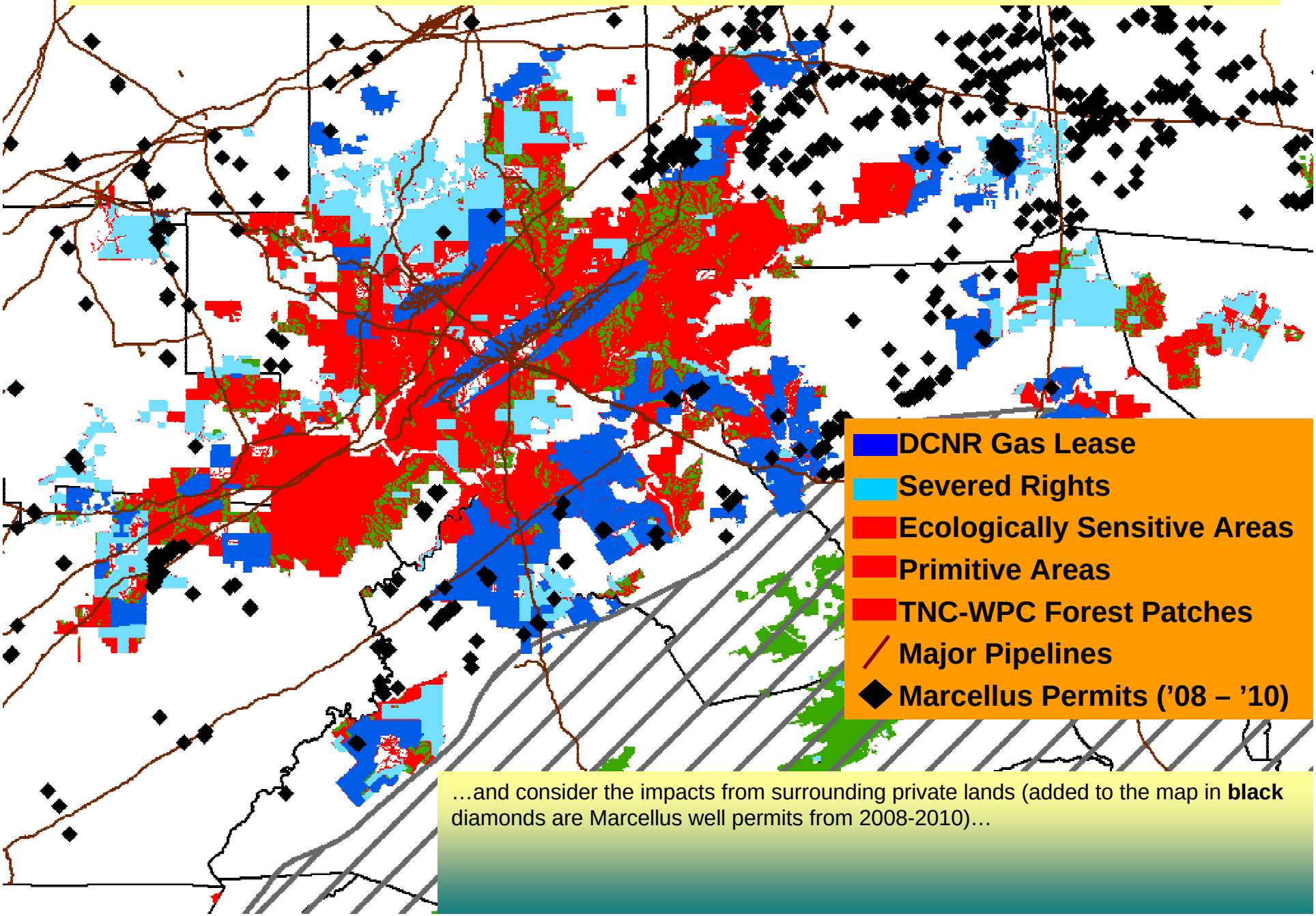

…along with Primitive areas (added in **red**)…

# Cumulative Assessment & Impacts



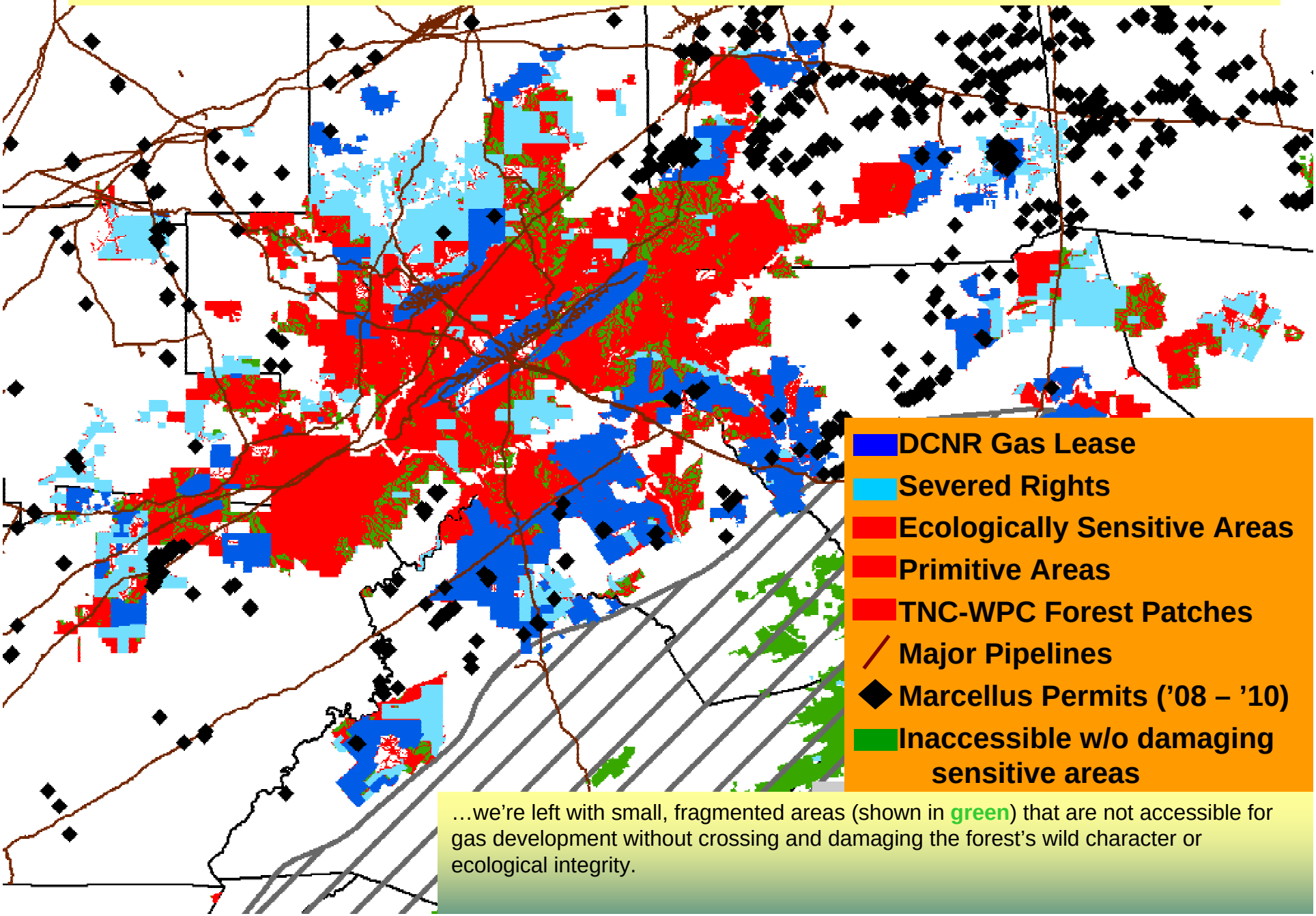

…and Priority Forest Patches (added in **red**) identified by The Nature Conservancy and The Western Pennsylvania Conservancy…

# Cumulative Assessment & Impacts



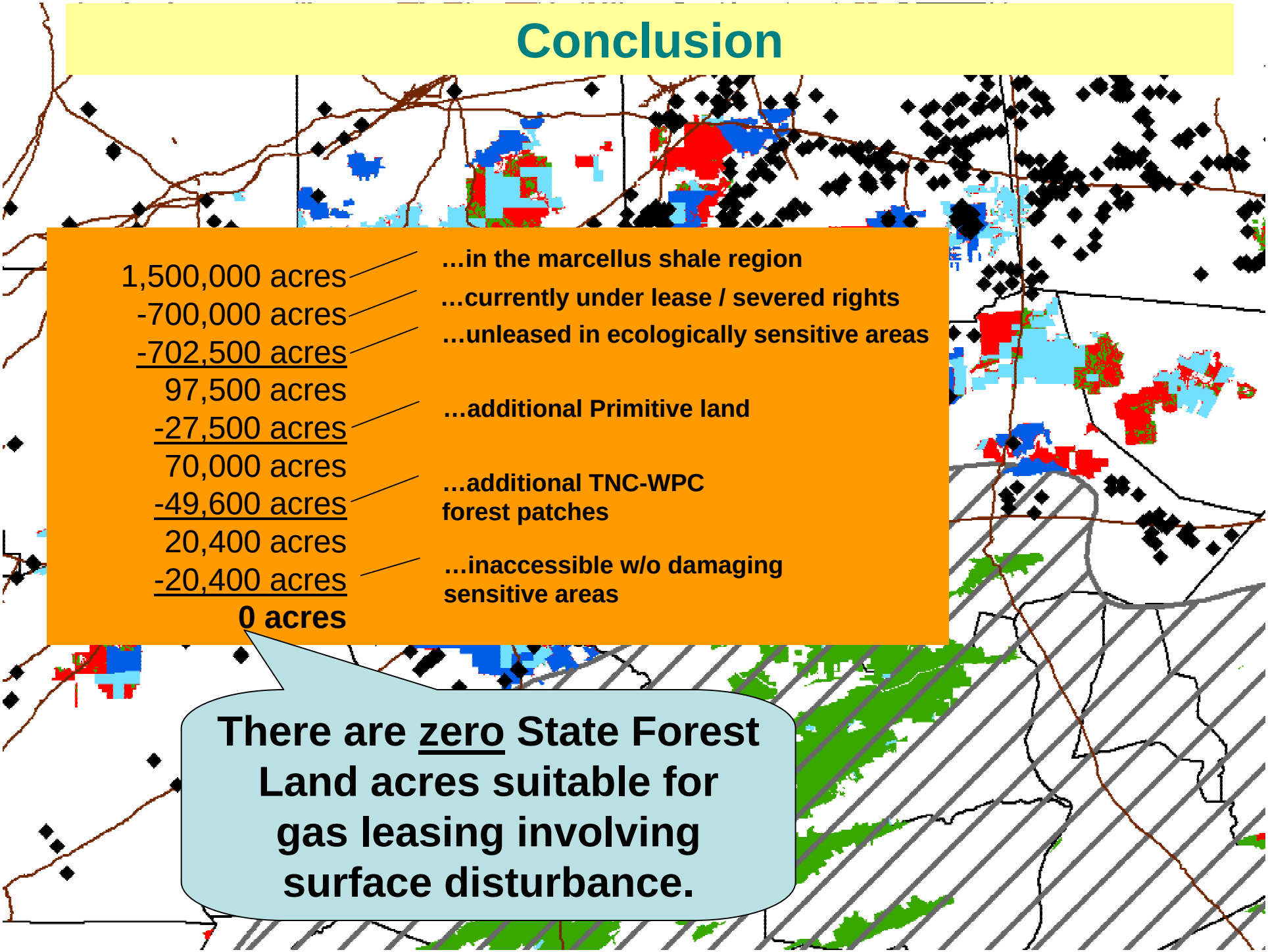

…and consider the impacts from surrounding private lands (added to the map in **black** diamonds are Marcellus well permits from 2008-2010)…

# Cumulative Assessment & Impacts




…we're left with small, fragmented areas (shown in **green**) that are not accessible for gas development without crossing and damaging the forest's wild character or ecological integrity.

# Conclusion



| | |
|---|---|
| 1,500,000 acres | …in the marcellus shale region |
| -700,000 acres | …currently under lease / severed rights |
| -702,500 acres | …unleased in ecologically sensitive areas |
| 97,500 acres | |
| -27,500 acres | …additional Primitive land |
| 70,000 acres | |
| -49,600 acres | …additional TNC-WPC forest patches |
| 20,400 acres | |
| -20,400 acres | …inaccessible w/o damaging sensitive areas |
| **0 acres** | |

**There are zero State Forest Land acres suitable for gas leasing involving surface disturbance.**

# Ohio Announces Tougher Permit Conditions for Drilling Activities Near Faults and Areas of Seismic Activity

*4/11/2014* *Ohio DNR* *in Oil and Gas*

**COLUMBUS, OH** – Today Ohio Department of Natural Resources (ODNR) Director James Zehringer announced new, stronger permit conditions for drilling near faults or areas of past seismic activity. The new policies are in response to recent seismic events in Poland Township (Mahoning County) that show a probable connection to hydraulic fracturing near a previously unknown microfault.

New permits issued by ODNR for horizontal drilling within 3 miles of a known fault or area of seismic activity greater than a 2.0 magnitude would require companies to install sensitive seismic monitors. If those monitors detect a seismic event in excess of 1.0 magnitude, activities would pause while the cause is investigated. If the investigation reveals a probable connection to the hydraulic fracturing process, all well completion operations will be suspended. ODNR will develop new criteria and permit conditions for new applications in light of this change in policy. The department will also review previously issued permits that have not been drilled.

“While we can never be 100 percent sure that drilling activities are connected to a seismic event, caution dictates that we take these new steps to protect human health, safety and the environment,” said Zehringer. “Not only will this reasonable course of action help to ensure public health and safety but it will also help us to expand our underground maps and provide more information about all types of seismicity in Ohio.”

“ODNR’s directives are a sensible response to a serious issue that regulators across the country are closely examining,” said Gerry Baker, Associate Executive Director of the Interstate Oil and Gas Compact Commission. “IOGCC is pleased to work with Ohio and other states to share scientific data to better understand the nature of these occurrences.”

“These additional standards add even more strength to Ohio’s already comprehensive regulatory program,” said Mike Paque, Executive Director of the Groundwater Protection Council. “State regulators are taking an aggressive lead in tackling tough and complicated oil and gas issues and ODNR is no exception.”

More than 800 wells have been drilled in Ohio’s Utica and Marcellus shale play, including as many as 16,000 hydraulic fracturing stages from those wells. Regarding the seismic events in Poland Township, Mahoning County, ODNR geologists believe the sand and water injected into the well during the hydraulic fracturing process may have increased pressure on an unknown microfault in the area. Further hydraulic fracturing at the site is suspended but the company will be permitted to recover resources from five of the previously drilled wells located on the pad. This is also expected to have the beneficial effect of reducing underground pressure and decreasing the likelihood of another seismic event.

Archive

- May 2014 (1)
- April 2014 (19)
- March 2014 (16)
- February 2014 (12)
- January 2014 (13)
- December 2013 (11)
- November 2013 (23)
- October 2013 (25)
- September 2013 (16)
- August 2013 (19)
- July 2013 (17)
- June 2013 (22)
- May 2013 (18)
- April 2013 (21)
- March 2013 (28)
- February 2013 (23)
- January 2013 (16)
- December 2012 (19)

News by Division

- Clean Lakes > (2)
- Coastal > (10)
- Engineering > (1)
- Fall Color > (7)
- Forestry > (42)
- Geological Survey > (2)
- Mineral Resources > (4)
- Natural Areas > (15)
- Oil and Gas > (7)
- Real Estate > (5)
- Soil and Water > (23)
- State Parks > (49)

Watercraft > (36)

Wildlife > (130)

Under ODNR's lead, Ohio has joined a consortium of state regulators dedicated to learning more about seismic activity, especially as it relates to oil and gas activity. The members of this consortium are currently working with the Interstate Oil and Gas Compact Commission and Groundwater Protection Council to share information and knowledge. The working group also hopes to draw upon current and future research to develop common procedures for how to monitor for seismic activity and respond if activity occurs.

The Ohio Seismic Network, coordinated by ODNR and operated by various partners, began recording seismic events in 1999. Before that time, the recording of seismic events varied from distant machines and felt reports. Ohio has a history of seismic activity, and since the network has established, Ohio has experienced 109 events greater than 2.0 magnitude. Data from the Ohio Seismic Network will be used as part of our new application review process.

A map of underground seismic faults and past seismic events is available at oilandgas.ohiodnr.gov.

*ODNR ensures a balance between wise use and protection of our natural resources for the benefit of all. Visit the ODNR website at ohiodnr.gov.*

Tags: Oil and Gas

Like 90 Tweet 56 g+1 0 25