7468 Dufferin Avenue
Riverside, CA 92504
(951) 687-4471
acwilson11@yahoo.com

February 18, 2016

VIA E-MAIL ONLY

Jeanine Townsend, Clerk to the Board
State Water Resources Control Board
1001 I Street, 24th Floor
Sacramento, CA 95814
E-mail: commentletters@waterboards.ca.gov

Karen Smith, MD, MPH
State Health Officer and Director
California Department of Public Health
1615 Capitol Avenue
Sacramento, CA 95814
E-mail: Karen.Smith@cdph.ca.gov

Re: Comment Letter – General Order for Recycled Water Use

Dear Ms. Townsend and Dr. Smith:

I would like to thank the State Water Resources Control Board ("State Board") for the opportunity to submit this comment letter in response to the above-referenced general order (the "Order"). I have a B.A. in chemistry from the University of Chicago and am a member of the State Bar, but my principal occupation is farming oranges. I have extensive experience in growing oranges. My family has been farming oranges in California since 1922. I am submitting this letter solely on behalf of myself, as an orange grower and member of the general public.

I believe the Order is overbroad and allows the use of recycled water that has not received adequate treatment to be safe. The Order could lead to adverse public health effects, and I accordingly object to it. I am also requesting a clarification of the factual and legal basis for the Order.

Water Code section 13521 requires the California Department of Public Health (CDPH) to adopt uniform state wide recycling criteria. The criteria, known as "title 22," authorize the use of disinfected tertiary treated water ("DTTR water") for crop irrigation. (22 CFR §60304.) I respectfully request the CDPH to provide the State Board and me a written response to the

following question: At the time the CDPH adopted 22 CFR §60304 in the year 2000, did the CDPH quantify the degree of likelihood of adverse health effects of perchlorate accumulation in crops? The importance of the CDPH's answer to that question is explained below.

The State Board's proposed Order authorizes all DTTR water produced in California to be used for the irrigation of all crops in California. The Order places no upper limit on the amount of perchlorate that is permissible in DTTR water. Under the Order, unlimited amounts of perchlorate are acceptable.

Perchlorate is toxic to human health. Perchlorate in irrigation water presents what is known as a "systemic" risk because it can be taken up by the tree in the irrigation water and concentrated *inside* the fruit, rendering the fruit poisonous. I previously submitted a written comment to the State Board that included four scientific articles (the "5-27-14 Comment"). As the State Board is aware, the public health issues raised by those articles formed the basis of a lawsuit I filed against the State Board entitled Andrew C. Wilson v. State Water Resources Control Board, Los Angeles Superior Court Case No. BS149632. In that lawsuit I challenged a prior order issued by the State Board that was the precursor to the current proposed Order. Pertinent documents from that case are attached to this letter as Exhibits 3 - 11. The case was heard by the Honorable James C. Chalfant, who denied my petition.

Although Judge Chalfant denied my petition, he stated that I had raised a public health issue that I should continue to pursue.

> "THE COURT: I think you have got to go the Department of Public Health. I'm not discounting the issue you have raised. It is an issue. I don't know where it goes. I don't know how important or significant it is, but public health is public health. It's an important thing. And, you know, **I'm not suggesting that you should drop this issue**, but I do think you've got to present it to the entity whose job it is to address this.
>
> "And I'm not saying the Water Board doesn't have a responsibility for public health. I think they do, but the primary entity that has that responsibility is the Department of Public Health, and **you should present it to them**." (Ex. 11 (Reporter's Transcript), p. 23, emphasis added.)

The question whether DTTR water has been adequately treated to be safe for crop irrigation is an open question that needs to be addressed. Judge Chalfant summarized the scientific articles I presented as follows:

> "Attached to Wilson's letter were four articles about perchlorate authored from 2006 to 2012. AR 623. Those articles can be summarized as follows.
>
> "Perchlorate reduces 'the functioning of the thyroid gland, and poor thyroid function is an important cause of developmental deficits and adult disease.' AR 719. In humans, the thyroid gland needs iodide to produce thyroid hormone. Id. A compound

known as NIS is responsible for transporting iodide into the thyroid gland. Perchlorate inhibits the ability of NIS to take up iodide. Id. The reduced transport of iodide suppresses the production of thyroid hormone. Id. Thyroid hormone is essential for normal brain development, body growth as well as for adult physiology. AR 719. Recent research indicates that thyroid hormone insufficiency in pregnant women is associated with cognitive deficits in the children. Id.

"There is concern that perchlorate-contaminated waters 'may represent a health risk both as sources of drinking water and irrigation water for food crops.' AR 688. Human exposures to perchlorate 'are likely attributed to both contaminated drinking water and food; in fact, a recent analysis concludes that a majority of human exposure to perchlorate comes from food.' AR 719.

"Perchlorate is not physically or chemically retained by soil (AR 690), and is largely transported into and through soils with irrigation water. AR 719. Perchlorate is chemically stable when wet. AR 719. The California drinking water safety limit for perchlorate is 6 parts per billion. AR 627. Perchlorate can be introduced into municipal sewers from waste discharge by industrial processes using perchloric acid. AR 676-68. Treated municipal wastewater can have perchlorate concentrations ranging from 250 parts per billion to 700 parts per billion. AR 678.

"Orange trees can have perchlorate levels that are higher than wastewater. This is because orange trees take up perchlorate with irrigation water, and the concentration in the orange fruit is higher than the concentration in the irrigation water. AR 690. This is because as water taken into a tree evaporates, salts are left behind and accumulate. AR 622. Orange trees in Loma Linda, California, irrigated with contaminated well water with a perchlorate level of 18 parts per billion produced oranges with a perchlorate level of 38 parts per billion. AR 692." (Ex. 6 (Decision on Writ) p. 5-6. See also Ex. 1, a factual background summary I have prepared, and Ex. 2, my 5-27-14 Comment, the evidence and arguments of which I urge against the current proposed Order.)

The treatment processes approved for DTTR water do not remove or reduce perchlorate. The current Order contains findings that correctly state that "[p]erchlorate accumulation has been documented in fruit and seed bearing crops and leafy vegetation irrigated with perchlorate contaminated water." (Order, p. 13.)

A "risk" exists when a reasonable person would recognize the possibility of an injurious event happening. A reasonable person would recognize that it is possible that DTTR water contains perchlorate that can be absorbed by roots and concentrated in a crop and adversely affect public health.

The crucial question is this: What are the chances that will happen? By chances I mean the degree of likelihood, or probability, that the event will occur. For example, the degree of likelihood that a harmful event will occur may be one million to one or a thousand to one.

The safety of DTTR water cannot be evaluated unless the degree of likelihood of adverse health consequences can be determined. Although Judge Chalfant denied my petition, he did not disagree with the basic principle I have been advocating:

> "MR. WILSON: Okay. The other argument I wanted to make, and I know I'm running out of time, I just want to say that the whole point of this testing of perchlorate, the whole principle I was trying to get across, was that a use of recycled water can't be considered safe if a reasonable person would recognize that the degree of likelihood of adverse public health effects can't be determined without further testing or further investigation. That's just the basic principle I was trying to bring home.
>
> "THE COURT: **I can't say that I disagree**." (Ex. 11, p. 42, emphasis added.)

When the State Board pronounces DTTR water to be safe, ordinary people, including farmers, believe that someone in the government must have quantified the degree of likelihood of adverse health effects and decided that the probability of harm is low enough to be acceptable. An official safety pronouncement tends to tamp down any thought of inquiry or investigation into safety that otherwise might arise in the minds of conscientious users. An official safety pronouncement encourages the use of recycled water.

A. Regulatory Gap In Agency Oversight

I am concerned that a regulatory gap may exist in the oversight exercised by the State Board and the CDPH. The safety of DTTR water cannot be evaluated unless the degree of likelihood of adverse health consequences can be determined. I am concerned that there is widespread belief this determination has been made when in reality no one has done so. This unacceptable result is due in part to mistake and lack of inter-agency consultation.

Specifically, I am concerned that the State Board is presuming that the CDPH quantified the degree of likelihood of adverse health effects of perchlorate in DTTR water at the time the CDPH adopted 22 CFR §60304 in the year 2000. Relying on that presumption, the State Board feels there is no need to repeat that work.

I believe such a presumption is wrong; I believe that the CDPH, when it adopted the regulations, did not at that time quantify the degree of likelihood of adverse health effects of perchlorate accumulation in crops. I want to clarify that with the CDPH. That is why I request a written response to the question posed above: At the time the CDPH adopted 22 CFR §60304 in the year 2000, did the CDPH quantify the degree of likelihood of adverse health effects of perchlorate accumulation in crops?

I urge the State Board not to adopt the Order until the response of the CDPH has been received in the record.

I also want to understand with certainty whether the State Board is making such a presumption. My question to the State Board is this: Does the State Board presume that the CDPH quantified the degree of likelihood of adverse health effects of perchlorate accumulation in crops at the time CDPH adopted 22 CFR §60304 in the year 2000? I cannot tell for certain the answer to that question from the text of the Order itself. The State Board's mode of analysis is not clear.

Judge Chalfant denied my writ petition based on the presumption that the CDPH did, in fact, considered perchlorate contamination of crops when it adopted the Title 22 regulations in the year 2000. Judge Chalfant found: "CDPH must be presumed to have done its job in issuing the regulation and considered all potential contaminants and uses of recycled water, including perchlorate contamination." (Ex. 6, p. 9.) Based on that presumption, Judge Chalfant concluded that my sole remedy was to ask the CDPH to re-visit its prior analysis, I had no right to require the State Board to address perchlorate, and the State Board was entitled to rely on title 22.

I think everyone would agree that such a presumption, as made by Judge Chalfant, is inappropriate if the State Board does not actually believe it. It is possible that the State Board does not actually believe that the CDPH considered perchlorate contamination of crops when it adopted the Title 22 regulations in the year 2000. I request that the State Board's current actual belief on this point be disclosed and reflected in the record.

Judge Chalfant ruled that the State Board cannot rely on title 22 if the State Board should "reasonably believe" that title 22 does not address the public health risk at issue. (Ex. 11, p. 39.) In that case, going to the CDPH is not my sole remedy, and the State Board, prior to adopting the Order, is required to quantify the likelihood of adverse public health effects from perchlorate contamination of crops.

B. The State Board Has Not Determined the Degree of Likelihood of Adverse Health Effects.

Nothing in the record shows that the State Board has determined the degree of likelihood of adverse health effects due to crop contamination from perchlorate in DTTR water.

In support of the Order, the State Board's web site references a "Fact Sheet," which addresses perchlorate as follows:

> **"Endocrine disruptors such as perchlorate may be present in disinfected recycled water, absorbed by fruit-producing trees, and concentrated on [sic] the fruits.**

Does this General Order contain any requirements to address perchlorate in recycled water? Recycled water uses proposed by an administrator's Recycled Water Program must meet the Uniform Statewide Recycling Criteria and any other standards set by the State or Regional Water Boards for protection of public health. *The Uniform Statewide Recycling Criteria was reviewed by an expert panel to determine whether it is sufficiently protective of public health for agricultural food crop irrigation*. Based on literature and monitoring data reviewed, recycled water is a relatively insignificant source of perchlorate based on type and volume of recycled water used for agricultural irrigation, and levels of perchlorate monitored in facilities that discharge to surface water.

"While there is no specific requirement addressing perchlorate in the General Order, it was considered in preparation of the General Order as documented in a staff memorandum addressing perchlorate occurrence in sources of agricultural water supplies. This memorandum is posted at: http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/requirements.shtml Based on literature and monitoring data reviewed, recycled water is a relatively insignificant source of perchlorate based on (1) type and volume of recycled water used for agricultural irrigation and (2) levels of perchlorate monitored in facilities that discharge to surface water (17 NPDES facilities out of 214 facilities, 12 out of 17 facilities are recycled water production facilities). (Ex. 12 (Fact Sheet), p. 7, italics added.)

The Fact Sheet wrongly suggests that perchlorate levels are safe because "[t]he Uniform Statewide Recycling Criteria was reviewed by an expert panel to determine whether it is sufficiently protective of public health for agricultural food crop irrigation." There is an implication that the "expert panel" concluded that compliance with title 22 is sufficient to make perchlorate levels safe. However, the written report of the expert panel shows that the panel never considered perchlorate. The report itself never mentions perchlorate. The report shows that the panel never attempted to determine the degree of likelihood of adverse health effects due to crop contamination from perchlorate in DTTR water. (The Fact Sheet in the preceding paragraph identifies the expert panel's report, which is entitled "Review of California's Water Recycling Criteria for Agricultural Irrigation" and is posted at: http://nwri-usa.org/cdph.ag.htm. Ex. 12, p. 6.)

The passage of the Fact Sheet quoted above also refers to a State Board staff memorandum addressing perchlorate (the "Staff Memorandum"). The Staff Memorandum states that staff reviewed the monitoring data from 214 major NPDES waste water treatment facilities. Of those 214 facilities, only 17 monitor for perchlorate in their effluent. The Staff Memorandum describes the perchlorate test results of those 17 facilities as follows:

"A review of from January 2011 – July 2014 indicates perchlorate is sometimes present. When measureable perchlorate is present, it is generally below 2 ug/L. One facility reported a perchlorate concentration of 10 ug/L in a single sample event. (That was the only perchlorate data available for that discharger.)" (Ex. 13 (Staff Memorandum), p. 2 (concentrations expressed as "ug/l" are equivalent to "ppb").)

It is unclear whether the State Board intends the above passage to mean that a level of 2 ug/l is okay for all crops, but a level of 10 ug/l might not be okay. The Staff Memorandum provides no analysis of that data with regard to crop safety.

Rather, the Staff Memorandum is directed at determining if irrigating with recycled water is a significant source of perchlorate contamination in the *environment*. (Ex. 13, p. 2) In other words, the memorandum addresses the issue of whether irrigating with recycled water will result in perchlorate contamination of receiving bodies of surface water or ground water. The memorandum concluded that irrigating with recycled water is a relatively insignificant source of perchlorate in the environment based on type and volume of recycled water used for agricultural irrigation, and levels of perchlorate monitored in facilities that discharge to surface water.

In addition, only 17 of the 214 major facilities test for perchlorate. A reasonable person would recognize that it is possible that one or more of the remaining 197 facilities have perchlorate levels that are higher than those 17 facilities. How can that be determined without testing? The likelihood that harm will occur from any particular one of those 197 facilities can only be determined by testing the effluent of the particular facility.

In general, for any given crop, the level of perchlorate in the edible portion of the crop, and the likelihood of harm to the public health, increases with increased levels of perchlorate in the irrigation water. The State Board needs to determine what is the likelihood of harm associated with different levels of perchlorate in the water. The determination needs to be based on science. The State Board needs to make sure that the actual perchlorate level in DTTR water does not result in an unacceptably high probability of harm.

Prior to declaring DTTR water safe, the State Board should (1) decide for different crops what upper level of perchlorate results in an acceptable probability of harm, and (2) require ongoing monitoring to see that those levels are not exceeded.

Testing water for perchlorate is not expensive. Babcock Laboratories, a local Riverside lab, charges $175 to test for perchlorate. The consequences of perchlorate toxicity to unborn children are significant. A reasonable person trying to prevent harm to the public health would test DTTR water for perchlorate prior to using it for crop irrigation. Rather than speculating that the perchlorate level is likely to be low, the level should simply be tested. The Order approves conduct, the use of un-tested DTTR water, that creates an unreasonable danger to public health.

I believe the State Board should consult with and receive in evidence the recommendations of CDPH on the health issues concerning perchlorate prior to adopting the Order. Among other things, the Order cites Water Code section 13523, which provides for consultation with CDPH, and consultation is envisioned by the Memorandum of Agreement

between the agencies. (Ex. 14.) I believe that the CDPH, including its Division of Food Drug and Radiation Safety, has expertise in systemic contamination of crops, and should not be totally shut out of the process.

The scientific articles I previously submitted contain information and data about the harmful effects of perchlorate that did not exist when title 22 was adopted in the year 2000. Prior to adopting the Order, the State Board should consider current science, including these articles, when determining the likelihood of adverse health effects from perchlorate contamination of crops. The perchlorate level in the edible portion of an orange can be significantly higher than the level in the irrigation water. A farmer's family member or loyal customers may drink a glass of orange juice from the farm every morning. The likelihood of adverse health effects from drinking orange juice with a perchlorate level in excess of the drinking water safety limit of 6 ppb should be a matter of concern.

C. Vague Alternative Grounds.

It appears that the State Board may be relying on new alternative and independent grounds to support a conclusion that perchlorate levels are safe. The proposed Order repeats verbatim most of the findings contained in the previous order of the State Board that was upheld by Judge Chalfant. However, additional language has been added to two critical findings. The italicized language has been added to the following finding:

> By restricting the use of recycled water to those meeting the Uniform Statewide Recycling Criteria *or other standards set by State Water Board and Regional Water Board for protection of public health*, this General Order ensures that recycled water is used safely. (Order, p. 11, italics added.)

The same new language has been added to this finding:

> When used in compliance with the Recycled Water Policy, the Uniform Statewide Recycling Criteria *or other standards set by State Water Board and Regional Water Board for protection of public health*, and all applicable state and federal water quality laws, the State Water Board finds that recycled water is safe for approved uses, and strongly supports recycled water as a safe alternative to raw and potable water supplies for approved uses. (Order, p. 3, italics added.)

The Order does not disclose what these "other standards set by State Water Board and Regional Water Board for protection of public health" are, and leaves the State Board's actual mode of analysis improperly vague and hidden from scrutiny. The matter needs clarification.

Sincerely,

Andrew C. Wilson

Andrew C. Wilson

TABLE OF EXHIBITS

EXHIBIT 1

ADDITIONAL BACKGROUND

DTTR water is "disinfected tertiary treated water." (22 CFR §60301.230.) The approved treatment processes for DTTR water set out in title 22 do not remove or reduce perchlorate. (Title 22 does not mention perchlorate.)

Standard treatments applied at sewer plants are generally referred to as primary, secondary, and tertiary. Primary stage treatment involves allowing solids to settle to the bottom, which helps reduce turbidity. "Turbidity" refers to the cloudy appearance of water due to tiny suspended solid particles. (When a child stirs up a mud puddle with a stick, the cloudy appearance or "turbidity" of the water is increased, and if the puddle is left alone, the solids will settle to the bottom, the water becomes more clear, and turbidity is reduced.)

Secondary treatment involves oxidation through bubbling oxygen or air through the water to create dissolved oxygen, which promotes the activity of microorganisms that break down organic matter, which helps reduce odors. Increasing dissolved oxygen does not reduce perchlorate levels.

Tertiary treatment means filtering the water to reduce turbidity to certain standards. Perchlorate does not exist in water as a suspended solid, rather it is a dissolved ion. Filtration to reduce turbidity to the applicable turbidity standards does not reduce perchlorate levels.

DTTR water has been oxidized and filtered to meet certain turbidity standards, and then "disinfected." Disinfection means reducing organisms that cause disease, or "pathogenic" organisms. Title 22 does not dictate the method of disinfection, but allows various methods. Disinfection may be accomplished by adding sodium hypochlorite, which is the active ingredient in household bleach. The concentration of sodium hypochlorite in household bleach is about 6% by weight, and up to about 16% by weight in commercial solutions used at sewer treatment plants. The disinfection process can actually introduce perchlorate into the water because sodium hypochlorite in storage can decompose to perchlorate, especially under warm conditions.

The take up and concentration of perchlorate in the edible portion of the crop varies among plant species. In addition, for a given plant species, the take up and concentration of contaminants such as perchlorate in the irrigation water also depends on climate. The transpiration of plants is much greater in hotter desert valleys than in the coastal region. Higher transpiration leads to higher concentration of contaminants inside the plant.

For orange trees, the concentration of perchlorate in the leaves is higher than in the edible portion of the fruit. This is due to the fact that greater transpiration occurs in the leaves. (In one study of oranges trees, the average perchlorate concentration in the fruit was 7.4 ppb while the average concentration in the leaves was 1,424 ppb. Ex. 2-C) The high transpiration rate through leaves is the concern with regard to perchlorate contamination of leafy vegetables.

EXHIBIT 2

7468 Dufferin Avenue
Riverside, California 92504
(951) 687-4471
acwilson11@yahoo.com

May 27, 2014

VIA E-MAIL ONLY

Jeanine Townsend, Clerk to the Board
State Water Resources Control Board
1001 I Street, 24th Floor
Sacramento, California 95814
E-mail: commentletters@waterboards.ca.gov

Re: Comment Letter – General Order WDRs for Recycled Water Use

Dear Ms. Townsend:

I would like to submit this comment letter in response to the above-referenced Order. I am a member of the State Bar, but my principal occupation is farming oranges. I am submitting this letter solely on behalf of myself, as an orange grower and member of the general public. I believe the Order is overbroad, and can lead to adverse public health effects, and I am requesting a clarification of the factual basis and reasons for the Order.

Under the Order, all municipal wastewater produced in California that is disinfected "tertiary" treated wastewater is approved for the irrigation of all the food crops grown in California. The standards for producing tertiary treated wastewater are set out in the regulations (Title 22). These standards do not set any limits on the level of endocrine disrupting chemicals in the end product. In other words, it is possible for a treated municipal wastewater to meet disinfected tertiary standards and still contain levels of endocrine disrupting chemicals that exceed drinking water safety limits.

Endocrine disrupting chemicals (EDCs) change the level of hormones in the human blood stream, and can be especially dangerous to pregnant women because hormone levels in the mother's blood regulate the development of the child. For example, perchlorate is an EDC that inhibits the production of thyroid hormone. Thyroid hormone insufficiency in an expecting mother alters the development of the child's brain, resulting in impaired cognitive ability. (Vandenberg 2012)

Orange trees can concentrate EDCs. For instance, orange trees grown at Loma Linda, California, irrigated with contaminated well water that had a perchlorate level of 18 ppb produced oranges having a perchlorate level of 38 ppb. (Sanchez 2006) The California drinking water safety limit for perchlorate is 6 ppb.

I cannot imagine that anyone, including the most ardent supporters of the Order, would urge a pregnant family member to drink a glass of orange juice from that Loma Linda grove every morning. The fruit from that orchard is not marketed commercially. (Sanchez 2006)

It is possible that tertiary treated municipal wastewater is being produced in California that has perchlorate levels similar to (or higher than) the Loma Linda well. Perchlorate can be introduced into municipal sewers as waste discharged from industrial processes that use perchloric acid. Perchlorate can also be introduced through the tertiary treatment process itself. Tertiary treatment often involves the use of sodium hypochlorite, which is the active ingredient in household bleach. Sodium hypochlorite in storage can decompose to perchlorate, especially under warm conditions. (MDEP 2006, Greiner 2008)

Does the State Board know of any reports or records that would show the different perchlorate levels of the various tertiary treated municipal waste waters being produced in this state?

I am concerned that the answer to the above question is "no." Yet the Order contains this finding: "By restricting the use of recycled water to title 22 requirements, this order ensures that recycled water is used safely." (Order, at p. 9.) I think it would have been more accurate if the Order had stated the facts to be: "There is a possibility that some tertiary treated recycled water that this Order authorizes for irrigation of oranges may contain perchlorate levels that are not safe. The State Board does not know what the perchlorate levels are in the different recycled waters, or which of the waters is safe, and does not require perchlorate levels to be tested." Is this an accurate statement of the facts?

Testing water for perchlorate is not expensive. Rather than speculating or arguing that perchlorate levels are likely to be low, or likely to be high, the levels should simply be tested.

I am concerned about other EDCs in addition to perchlorate. Human hormones are active at extremely low blood concentrations, some as low as parts per trillion. Hormones regulate gene expression – the process of transcription and translation of an individual's DNA. Introducing into the blood even small amounts of EDCs that mimic these hormones can modify gene expression. (Vandenberg 2012)

Perchlorate is an example of only one EDC that is known to be potentially present in tertiary treated wastewater. Many are toxic man-made chemicals used in industrial processes or released as waste products from industrial processes. Perchlorate is a regulated EDC. There are unregulated EDCs and other toxic chemicals know to be potentially present in tertiary treated water, including perflourocarbons and the constituents of emerging concerns (CECs).

When routed directly from a sewage plant to a crop these toxic chemicals have not been in the ground for six months or undergone any similar attenuating process.

The public health issue presented by EDCs in crop irrigation water can be illustrated by the fate of naturally occurring chemicals in irrigation water. These include "salts" which is a broad term that generally refers to various chemical constituents that have diverse effects upon the soil and tree.

Salt chemicals in the irrigation water are absorbed by the roots and taken up in the tree with the irrigation water. As water evaporates from the tree into the air, the salts are left behind and accumulate in the tree. This is how salts can have higher concentration levels in plant tissues than in the irrigation water.

For citrus the absorption process generally follows certain principles. The higher the salt content of the irrigation water, the higher the accumulation in the tree. Trees grown in cooler coastal locations accumulate less than trees grown in the hotter drier inland valleys. Trees accumulate less during cooler, overcast summers than during hot summers. Citrus trees are budded onto various citrus rootstocks. The different rootstocks vary in the amount of salt they accumulate.

Absorption through plant roots is the operating principle of the so-called "systemic pesticides." Systemic pesticides are chemicals added to the irrigation water and taken up by the plant through the roots. The pesticides are in the sap of the plant. When the insect bites into the plant and ingests the sap it dies. Prior to government approval, these chemicals are tested and data collected to measure or quantify the degree of likelihood that adverse public health effects will occur, and safe application rates and timing are established.

Normally in analyzing risk there are two distinct factual issues 1) Is it possible for an injurious event to happen, and 2) what is the degree of likelihood that the event will or will not occur?

Has the State Board made a factual determination as to whether it is possible that EDCs or other toxic chemicals potentially present in tertiary treated wastewater can be absorbed by roots and into a crop and adversely affect public health? I urge the Board to clarify the Order to disclose the Board's decision on this issue with regard to each chemical and each crop the Board has considered.

If the Board has decided that an adverse public health effect is possible, has the Board made a factual determination as to the degree of likelihood that the effect will occur? I urge the Board to clarify the Order to disclose the Board's decision on this issue with regard to each chemical and each crop the Board has considered.

One of the primary statutory conditions on the use of recycled water is the protection of public health. Until these factual issues are decided, there is no factual basis to support a conclusion that the Order protects the public health.

References

Copies of the references cited in this letter are attached as the following exhibits:

Greiner 2008 (Exhibit A)
MDEP 2006 (Exhibit B)
Sanchez 2006 (Exhibit C)
Vandenberg 2012 (Exhibit D)

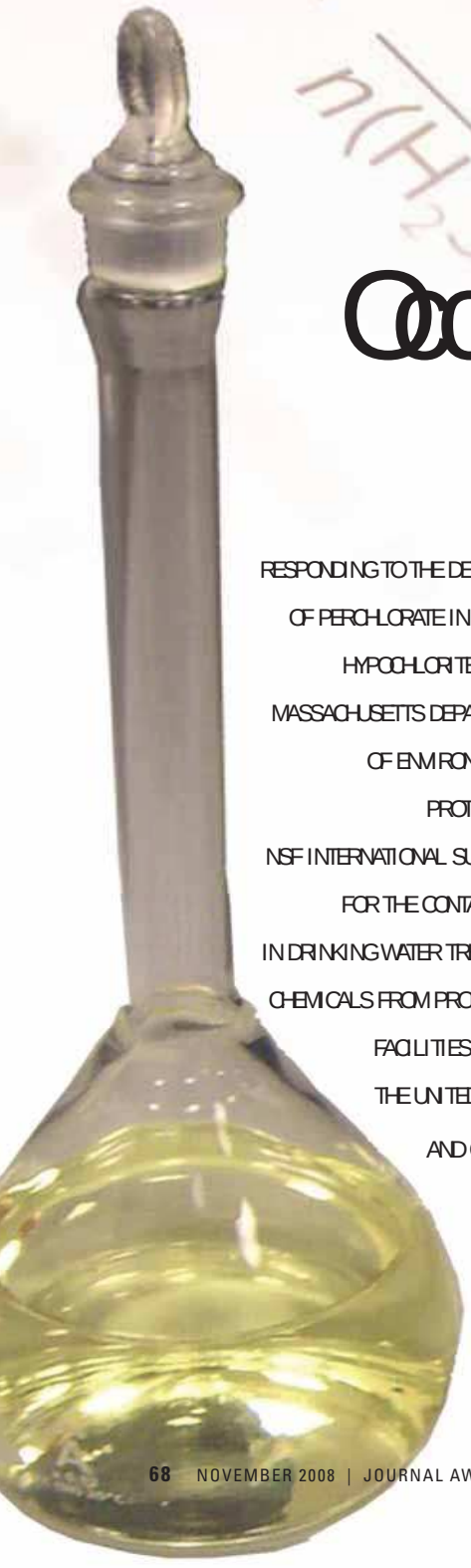
Respectfully submitted,

Andrew C. Wilson

Andrew C. Wilson

EXHIBIT A

BY PETER GREINER, CLIF MCLELLAN, DALE BENNETT, AND ANGIE EWING



Occurrence of perchlorate in sodium hypochlorite

RESPONDING TO THE DETECTION OF PERCHLORATE IN SODIUM HYPOCHLORITE BY THE MASSACHUSETTS DEPARTMENT OF ENVIRONMENTAL PROTECTION, NSF INTERNATIONAL SURVEYED FOR THE CONTAMINANT IN DRINKING WATER TREATMENT CHEMICALS FROM PRODUCTION FACILITIES ACROSS THE UNITED STATES AND CANADA.

Perchlorate is both a synthetic and a naturally occurring chemical. Most of the perchlorate that is manufactured in the United States is used as the primary ingredient of solid rocket propellant. Wastes from the manufacture and improper disposal of perchlorate-containing chemicals are increasingly being discovered in soil and water (USEPA, 2007).

An additional source of perchlorate in drinking water has been found to occur through the use of sodium hypochlorite. The Massachusetts Department of Environmental Protection (MDEP) has reported that significant levels of perchlorate can be detected in sodium hypochlorite samples that have aged for a few weeks (MDEP, 2005). Sodium hypochlorite as delivered to one utility had a perchlorate concentration of 0.2 µg/L in the product, but the level of perchlorate rose to 6,750 µg/L after the product had aged for 26 days.

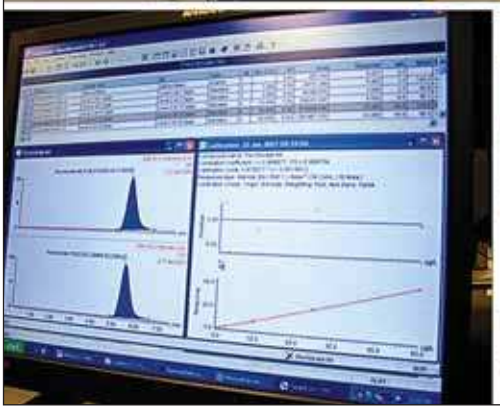
INVESTIGATION OF WATER TREATMENT CHEMICALS BEGAN IN 2005

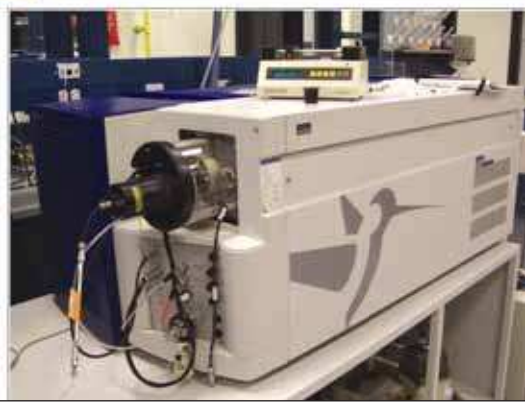
In 2005 NSF International began analyzing samples of drinking water treatment chemicals for the contaminant perchlorate. These samples were collected as part of the annual testing requirement to support NSF certification of the treatment chemical to NSF/American National Standards Institute Standard 60: Drinking Water Treatment Chemicals—Health Effects (NSF/ANSI, 2005). Samples collected included not only sodium hypochlorite but other types of chemicals as well. NSF 60 currently requires testing of sodium hypochlorite samples for regulated metals, volatile organic compounds, and bromate.

NSF continued the investigation of sodium hypochlorite through July 2006, resulting in the analysis of more than 67% of NSF-certified manufacturers across North America. The levels of perchlorate reported here reflect potential at-the-tap concentrations calculated in accordance with the proce-

Aliquots of the sodium hypochlorite samples collected at manufacturers' facilities were placed in 40-mL amber glass vials and stored in the dark prior to testing.

Perchlorate concentrations were determined by a liquid chromatography/mass spectrometry technique based on US Environmental Protection Agency method 331.0.





dures in NSF 60. These "normalization" calculations project potential at-the-tap concentrations by assuming the treatment chemical is dosed at the maximum use level (MUL) for which it was certified. Typically the MUL for sodium hypochlorite products is equivalent to dosing 10 mg/L of total chlorine into water. Although this concentration is significantly above the US Environmental Protection Agency (USEPA) maximum residual disinfectant level goal of 4.0 mg/L, it provides a worst-case evaluation of the sodium hypochlorite by accounting for other potential uses such as prechlorination during water treatment and use during shock chlorination of water systems.

Perchlorate health effects. Perchlorate affects the ability of the thyroid gland to take up iodine (ATSDR, 2005). Iodine is needed to make thyroid hormones that are released into the blood and regu-

late many body functions. Perchlorate is considered harmful to health when its inhibition of iodine uptake is great enough to affect the thyroid. There is concern that human exposure to higher amounts of perchlorate for a long time may lower the level of thyroid activity and lead to hypothyroidism. Low levels of thyroid hormones in the blood may adversely affect the skin, cardiovascular system, pulmonary system, kidneys, gastrointestinal tract, liver, blood, neuromuscular system, nervous system, skeleton, male and female reproductive systems, and numerous endocrine organs. Studies in animals have shown that the thyroid gland is the main target of perchlorate toxicity. Animal studies provided inconclusive results regarding effects of perchlorate on the immune system. Perchlorate did not affect reproduction in rats, according to one study.

Perchlorate is considered harmful to health when its inhibition of iodine uptake is great enough to affect the thyroid.

Perchlorate regulation and guidance criteria. In October 2008 the USEPA announced a preliminary determination on the regulation of perchlorate. After conducting an extensive review of scientific data related to the health effects of exposure to perchlorate from drinking water and other sources, USEPA ". . . found that in over 99% of public drinking water systems, perchlorate was not at levels of public health concern. Therefore, based on the Safe Drinking Water Act criteria, the agency determined there is not a 'meaningful opportunity for health risk reduction' through a national drinking water regulation" (USEPA, 2008). USEPA will make a final determination for perchlorate after a 30-day public comment period. The agency also intends to issue a health advisory at the time it issues the final regulatory determination in order to assist states with their local response for perchlorate.

At the state level, perchlorate guidance criteria of 14 µg/L in Arizona, 5 µg/L in New York, and 1 µg/L in Maryland and New Mexico have been adopted, along with action levels of 18 µg/L in New York and Nevada and 4 µg/L in Texas (Bull et al, 2004). California has established a perchlorate maximum contaminant level (MCL) of 0.006 mg/L (CDPH, 2007), and Massachusetts has established a perchlorate MCL of 0.002 mg/L (MDEP, 2006). For the purposes of estimating the effect of perchlorate contamination, the current research used the lowest of these values, in other words, 1 µg/L.

SAMPLES NORMALLY COLLECTED DURING UNANNOUNCED AUDITS TESTED FOR PERCHLORATE

As part of NSF's certification program for drinking water treatment chemicals, unannounced audits of manufacturing sites are performed annually, and samples of certified treatment chemicals are taken from recent production or retains. NSF used portions of these normally collected samples for this research on perchlorate. Once the samples were received at NSF, aliquots were placed in 40-mL amber glass vials and stored in the dark at room temperature before testing.

Laboratory analysis. The analysis for perchlorate was performed according to a modified USEPA method 331.0, Determination of Perchlorate in Drinking Water by Liquid Chromatography Electrospray Ionization Mass Spectrometry (USEPA, 2005). Method 331.0 is a method for analyzing drinking water. All method requirements relevant to the analysis of sodium hypochlorite rather than drinking water were included; the modification of this method at NSF related to modification of the quality control requirements.

Method 331.0 allows for identification by either tandem mass spectrometry mode or single ion monitoring mode using dual ions (masses 99 and 101). In this research, quantification was performed by internal standard calibration using the mass 101 ion. Results were reported in µg/L for liquid samples. In sodium hypochlorite, the average detection level for perchlorate was 250 µg/L.

Approximately one third of the samples tested were additionally tested on multiple days to determine the rate of change in perchlorate concentration as the sodium hypochlorite aged. Samples were maintained in the dark and at room temperature between analysis days.

164 CHEMICAL SAMPLES TESTED

Through July 2006, perchlorate testing was performed on 164 samples of drinking water treatment chemicals collected from 102 manufacturing locations. Of the 37 types of chemicals tested, perchlorate was detected in only two: sodium hydroxide and sodium hypochlorite (Table 1).

Of the 27 sodium hydroxide samples, 22 (81%) had perchlorate levels reported as nondetectable; in the remaining five samples, perchlorate concentrations ranged from 0.01 to 0.12 µg/L (Table 2).

The occurrence of perchlorate in sodium hypochlorite was a more common finding. Perchlorate was detected in more than 91% of the

samples tested, at levels ranging from 0.03 to 29 µg/L. Table 3 groups the results by concentration range, including a running average of samples containing perchlorate at levels less than or equal to the level of perchlorate in the range.

Of greater significance was the correlation between the age of the sodium hypochlorite and the level of perchlorate detected. Figure 1 shows the results of testing on samples with a known date of manufacture. Results, plotted by sample age at the time of analysis, clearly demonstrated a trend of increasing perchlorate concentration as the hypochlorite product aged.

Three of the samples tested yielded perchlorate concentrations of 8.8, 11, and 29 µg/L, significantly greater than the levels found in other samples; the 29-µg/L value does not appear in Figure 1 because the date of manufacture had not been established. Because these concentrations were significantly outside the observed levels of perchlorate formation in the other sodium hypochlorite samples tested, the authors believe that contamination of one of the component materials used to manufacture the sodium hypochlorite may be the primary perchlorate source.

Table 4 summarizes the occurrences of perchlorate by sodium hypochlorite age range. All of the samples tested within the first 30 days of production had a normalized perchlorate concentration below 1 µg/L. Of those samples tested between 30 and 45 days after production, 97% had perchlorate concentrations below 1 µg/L and just 3% had levels exceeding that value. Between 45 and 60 days after production, however, 8% of samples tested showed perchlorate concentrations exceeding 1 µg/L, and by 90 days after production, perchlorate levels in 84% of samples exceeded 1 µg/L.

Twenty-three of the samples tested were analyzed for perchlorate content on multiple days to provide insight into the rate of increase.

TABLE 1 Summary of samples tested by chemical type

Chemical	Samples—*n*	Samples With Perchlorate Detected—*n*	Samples With Perchlorate Detected %
Aluminum chloride	1		
Aluminum sulfate	2		
Ammonium hydroxide	3		
Bentonite	1		
Calcium hydroxide	1		
Calcium hypochlorite	2		
Calcium oxide	2		
Carbon dioxide	1		
Copper sulfate	2		
Ferric chloride	2		
Ferric sulfate	2		
Ferrous chloride	1		
Ferrous sulfate	1		
Fluorosilicic acid	1		
Fluosilicic acid	1		
Hydrochloric acid	1		
Hydrofluosilicic acid	1		
Hydrogen peroxide	1		
Phosphoric acid	3		
Polyaluminum silicate sulfate	1		
Potassium carbonate	1		
Sodium bicarbonate	1		
Sodium bisulfite	2		
Sodium carbonate	1		
Sodium chloride	2		
Sodium chlorite	1		
Sodium fluoride	1		
Sodium hexametaphosphate	1		
Sodium hydroxide	27	5	19
Sodium hypochlorite	82	75	91
Sodium polyphosphates, glassy	3		
Sodium silicate	5		
Sodium trimetaphosphate	1		
Sulfuric acid	2		
Trichloroisocyanuric acid	1		
Zinc chloride	1		
Zinc orthophosphate	2		
Total	164		

Samples were maintained in the dark and at room temperature between analyses. Results of the "over time" analysis are shown in Figure 2. The plots demonstrated a consistent rate of increase across multiple sample sources.

Portions of three of the sodium hypochlorite samples that were tested over time were diluted at a ratio of 1:2 with deionized water and also tested over time to determine whether the rate of perchlorate formation was significantly different in diluted form. As shown in Figure 3, a comparison of the full-strength and diluted samples found that the full-strength sodium

TABLE 2 Perchlorate occurrences in sodium hydroxide samples

Samples—*n*	Perchlorate in Chemical—*µg/kg*	Perchlorate At the Tap—*µg/L*
22	ND (250)	ND (0.03–0.05)
1	700	0.07
1	900	0.09
1	600	0.12
1	160	0.03
1	110	0.01

n—number, ND—not detected

ND results were below the detection level of the analytical procedure as identified in the parentheses. For calculation of the values in column 3, the level of perchlorate found in the chemical was multiplied by the maximum use level (MUL) certified for the individual chemical. Not all sodium hydroxides have the same certified MUL.

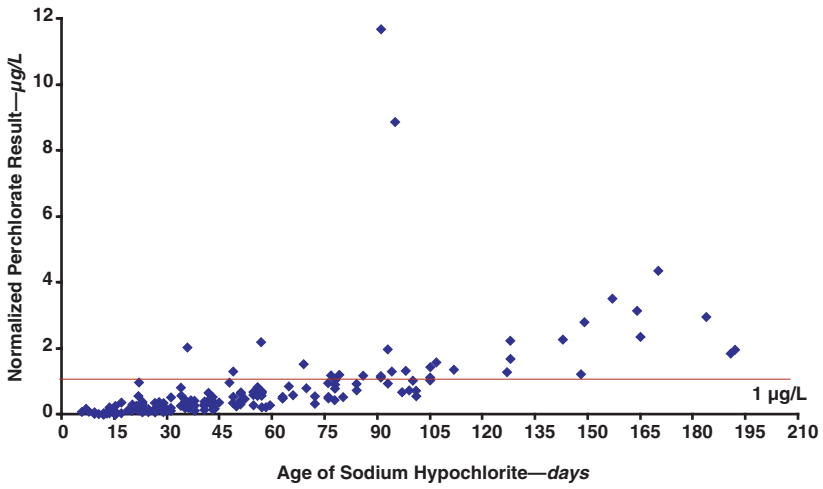
TABLE 3 Perchlorate concentration range in sodium hypochlorite samples*

Concentration Range—*µg/L*	Samples—*n*	Samples—%	Samples—Running %
ND	7	9	9
> ND–1.0	42	51	60
> 1–2	9	11	71
> 2–3	15	18	89
> 3–4	4	5	94
> 4–5	2	2	96
> 5–6	0	0	96
> 6–7	0	0	96
> 7–8	0	0	96
> 8	3	4	100
Total	82	100	

n—number, ND—nondetected

*At-the-tap in µg/L

FIGURE 1 Perchlorate in sodium hypochlorite (normalized to at-the-tap values)



hypochlorite generated perchlorate at a rate six to nine times faster than the same product diluted to half strength.

Three of the sodium hypochlorite samples were also evaluated over time to determine whether the level of bromate, chlorate, or chlorite also changed with age. No significant trend was noted for increasing or decreasing bromate levels. This was expected because almost all of the bromine in chlorine and the bromide in sodium hydroxide—the primary ingredients in sodium hypochlorite—are quickly converted to bromate at the pH of sodium hypochlorite (Chlorine Institute, 2004). The levels of chlorate and chlorite generally increased with age, but separate research is needed to better quantify that behavior.

Several factors were identified as contributing to variability in these results.

- Composite samples were collected from manufacturers across one or more days of the manufacturer's production retains. For the purposes of this study, the "date of manufacture" corresponding to these samples was the date of the earliest retain of the composite sample. This practice particularly affected the precise correlation between age of the sodium hypochlorite and the corresponding perchlorate level.
- The way the samples were stored and shipped to NSF prior to storage and analysis at NSF also added to the variability, given that both temperature and light have been reported to affect the rate of perchlorate formation.
- Results were normalized to the maximum use level (MUL) for the chemical in the NSF listing. The MULs were not necessarily proportional to the strength of the sodium hypochlorite nor were they directly associated with the level of chlorate. The levels of perchlorate in this study have been presented as potential at-the-tap levels because this was the

primary concern being addressed through NSF 60 evaluations.

SUMMARY AND CONCLUSION

Testing affirmed the recurrent presence of perchlorate in sodium hypochlorite. This appeared to be associated with the natural formation of perchlorate from chlorate, but results suggested there may also be occurrences of perchlorate attributable to contamination from component ingredients or manufacturing processes.

The data compiled by NSF to date supported the data previously collected by MDEP on perchlorate occurrence in sodium hypochlorite. The data also supported the MDEP's conclusion that the perchlorate levels were probably not a concern for most water utilities that use sodium hypochlorite within a few weeks of production. However, perchlorate occurrence may be a concern for water systems that store sodium hypochlorite for longer periods or have residual levels of aged chemical in storage tanks that may contaminate new shipments.

The data further indicated that NSF 60 should address perchlorate contamination. Perchlorate should be a required parameter for all sodium hypochlorite products, and a single product allowable concentration for perchlorate needs to be established in the standard. In addition, the data suggested a need for expiration dates on all sodium hypochlorite shipments to water utilities as well as on small containers of bleach that may be used by small systems.

For utilities that routinely use sodium hypochlorite supplies within 45 days of manufacture, the contribution of perchlorate is likely to be negligible unless there is some contamination of the original ingre-

TABLE 4 Perchlorate summary by age of NaOCl

Age of NaOCl at Testing *days after manufacture*	Analysis—*n*	Perchlorate		
		> 1 µg/ L—*n*	< 1 µg/ L %	> 1 µg/ L %
≤ 30	53	0	100	0
> 30 to ≤ 45	32	1	97	3
> 45 to ≤ 60	25	2	92	8
> 60 to ≤ 90	24	4	83	17
> 90	32	27	16	84
Total	166			

n—number, NaOCl—sodium hypochlorite

FIGURE 2 Perchlorate levels in sodium hypochlorite (normalized to at-the-tap values)

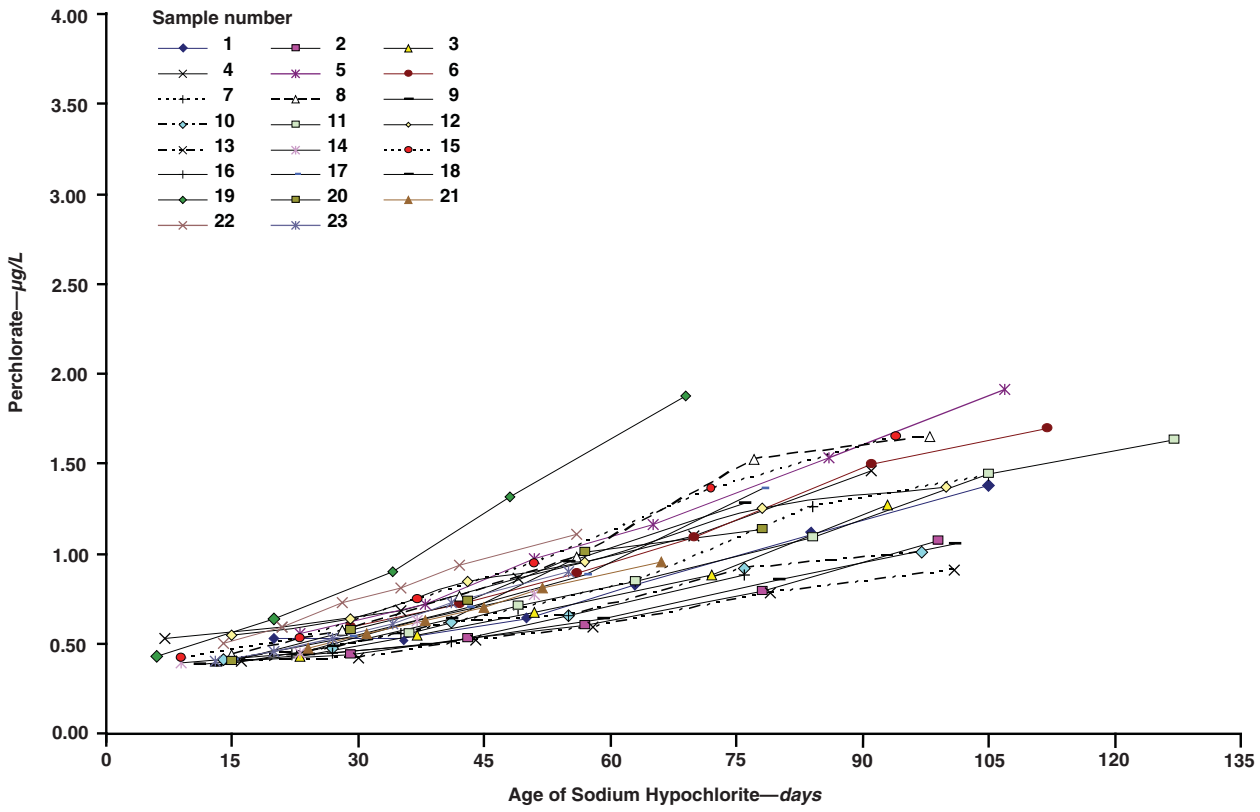
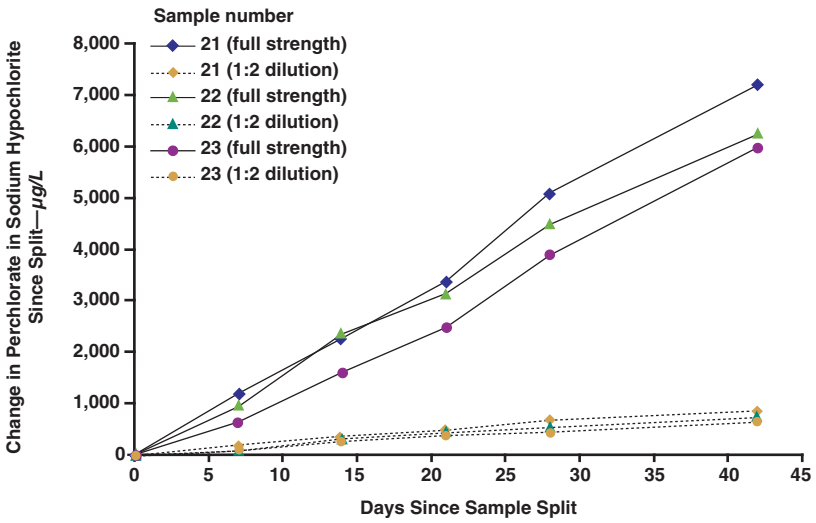


FIGURE 3 Comparison of perchlorate formation rates for full-strength and diluted (1:2 ratio) sodium hypochlorite



dients. Utilities or small systems that store sodium hypochlorite for longer periods may encounter significant levels of perchlorate in the finished drinking water. To minimize the perchlorate risk, sodium hypochlorite should be stored in the dark at cool temperatures, diluted if possible, and used within a few weeks of manufacture. Storage tanks and piping should also be emptied of aged material and flushed to minimize the potential for contamination.

ACKNOWLEDGMENT

The authors acknowledge the work performed by the state of Massachusetts and thank the staff of the California Department of Health Services for first bringing the issue to their attention.

ABOUT THE AUTHORS

Peter Greiner *(to whom correspondence should be addressed) is a technical manager with NSF International, 789 N. Dixboro Rd., Ann Arbor, MI 48105; greinerp@nsf.org. He received a BS degree in aquatic biology from Eastern Michigan University in Ypsilanti. Greiner has more than 28 years of experience in product certification and is currently a member of the Drinking Water Additives Joint Committee charged with oversight of NSF International/American National Standards Institute Standard 60. Clif McLellan is the director of toxicology services, Dale Bennett is an operations manager, and Angie Ewing is a senior toxicologist with NSF International.*

Date of submission: 06/20/07
Date of acceptance: 01/24/08

If you have a comment about this article, please contact us at journal@awwa.org.

REFERENCES

ATSDR (Agency for Toxic Substances and Disease Registry), 2005. Perchlorates. Div. of Toxicology & Envir. Med. ToxFAQs™, September. http://www.atsdr.cdc.gov/tfacts162.pdf.

Bull, R.J.; Chang, A.C.; Cranor, C.F.; Shank, R.C.; & Trussell, R., 2004. Perchlorate in Drinking Water: A Science and Policy Review. Urban Water Research Center, University of California, Irvine.

CDPH (California Department of Public Health), 2007. Perchlorate in Drinking Water. http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Perchlorate.aspx., accessed Nov. 17, 2007.

Chlorine Institute, 2004. Bromate in Sodium Hypochlorite Potable Water Treatment. Chlorine Institute, Arlington, Va., http://www.chlorineinstitute.org/files/FileDownloads/BromateinNaOCl-PotableWaterTreatment.pdf.

MDEP (Massachusetts Department of Environmental Protection), 2006. 7/28/06 Revisions to 310 CMR 22.00. Inorganic Chemical Maximum Contaminant Levels, Monitoring Requirements, and Analytical Methods. http://www.mass.gov/dep/water/laws/perchlorate-310CMR22-07282006.doc.

MDEP (Massachusetts Department of Environmental Protection), 2005. The Occurrence and Sources of Perchlorate in Massachusetts. Draft Report, http://www.mass.gov/dep/cleanup/sites/percsour.pdf.

NSF/ANSI (NSF International/American National Standards Institute), 2005. NSF/ANSI Standard 60. Drinking Water Treatment Chemicals—Health Effects. NSF Intl., Ann Arbor, Mich.

USEPA (US Environmental Protection Agency), 2008. Regulatory Determinations for Priority Contaminants on the Second Drinking Water Contaminant Candidate List. October 2008 Preliminary Regulatory Determination for Perchlorate. http://www.epa.gov/safewater/ccl/reg_determine2.html-perchlorate.

USEPA, 2007. Drinking Water Contaminants. Perchlorate. http://www.epa.gov/safewater/contaminants/unregulated/perchlorate.html.

USEPA, 2005. Method 331.0—Determination of Perchlorate in Drinking Water by Liquid Chromatography Electrospray Ionization Mass Spectrometry. EPA 815-R-05-007, Cincinnati. http://www.epa.gov/safewater/methods/pdfs/methods/met331_0.pdf.

EXHIBIT B

Massachusetts Department of
Environmental Protection

DRAFT REPORT

The Occurrence and Sources of Perchlorate in Massachusetts

August 2005

Updated April 2006

Massachusetts Department of Environmental Protection
1 Winter Street
Boston, MA 02108
http://www.mass.gov/dep/

This page intentionally blank

Executive Summary

In recent years, the Massachusetts Department of Environmental Protection (MassDEP) has undertaken a series of initiatives and studies to ascertain the extent to which the perchlorate ion is present in the groundwater and surface waters of the state. While many questions remain, based upon the totality of information obtained to date, the agency has made a number of preliminary findings and conclusions:

Occurrence

The perchlorate ion is not pervasive in surface water or groundwater in Massachusetts, having been found in only 9 of 600 tested public water supply systems at or above an analytical Reporting Limit of 1 μg/L (ppb). Detections have in most cases been related to known or suspected uses or releases of perchlorate-containing materials.

Sources

The most prevalent sources of perchlorate contamination in environmental media in Massachusetts were found to be blasting agents, military munitions, fireworks, and, to a lesser extent, hypochlorite (bleach) solutions. Additionally, at one location, a perchloric acid user was identified as a significant source of perchlorate contamination to a river system.

Impacts

The order-of-magnitude impacts associated with observed sources to date include:

- *Blasting agents* - hundreds to thousands of μg/L (ppb) in groundwater and small streams
- *Military Munitions* - hundreds of μg/L (ppb) in groundwater
- *Fireworks* - single digit to double digit μg/L (ppb) in groundwater
- *Industrial Perchloric Acid Use* - hundreds of μg/L (ppb) in effluent from municipal sewage treatment plant; single to double digit μg/L (ppb) in receiving river systems

Based upon a limited sampling effort, hypochlorite solutions used at water and wastewater treatment plants were found to contain between 260 and 6750 μg/L (ppb) of perchlorate, with concentrations of perchlorate increasing with time of product storage. This could result in detectable levels of perchlorate (0.2 – 0.4 μg/L) in chlorinated drinking water distribution systems. Perchlorate was also found in household bleach, from 89 μg/L (ppb) to 8000 μg/L (ppb), with concentrations increasing with time of product storage. While the on-site discharge of household bleach via washing machine use could result in low-level impacts to groundwater, discharges of perchlorate to conventional (anaerobic) septic tanks were found to be treated to less than 1 μg/L (ppb).

Analytical

The use of a modified EPA Method 314.0 was shown to reliably detect and quantify 1 μg/L (ppb) or greater concentrations of the perchlorate ion in drinking water matrices common in Massachusetts (i.e., less than 500 μS/cm specific conductance).

This page intentionally blank

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

Perchlorate is of concern because of its toxicity. It interferes with iodide transport into the thyroid gland, decreasing the availability of iodide needed for the synthesis of thyroid hormones, and thus has the potential to affect metabolism and normal growth and development, which could result in brain damage. The impacts of disrupting thyroid hormone synthesis are greatest on pregnant women and their developing fetuses, infants, children, and individuals who have low levels of thyroid hormones. More information in this regard is available from MassDEP at http://www.mass.gov/dep/brp/dws/percinfo.htm

Little is known about the prevalence of perchlorate in the environment, particularly at low concentrations. This is due in large part to the relatively recent introduction of mass-produced perchlorate-containing products to commercial and industrial marketplaces, combined with historical limitations in analytical testing technologies.

In an effort to shed some light on this subject, MassDEP has over the last 12 months initiated a series of investigatory efforts and programs. The purpose of this report is to explain and document these activities, and provide and discuss data and preliminary findings.

2.0 BACKGROUND

2.1. Production and Uses of Perchlorate

The unusual and desirable properties of Perchloric acid and perchlorate salts were first discovered in the early part of the 20th century. Both are powerful oxidizing agents that are also exceptionally stable and safe to use. (Schumacher, 1960)

The large-scale production of perchlorate salts began in the 1940s for military purposes, and in the following decades, for use as a solid oxidant in rockets and missiles. The two most common salts are ammonium and potassium perchlorate. To this day, the defense industry and NASA remain the largest users of perchlorate in the United States. According to the Department of Defense, perchlorate is currently used in over 250 types of munitions. (http://www.dodperchlorateinfo.net/facts/uses-benefits/)

Given this history and status, it is not surprising that concern over releases of perchlorate to the environment has focused on large perchlorate manufacturing and use facilities located in the western US, as well as military installations throughout the nation – including Massachusetts. However, in recent years it has become apparent

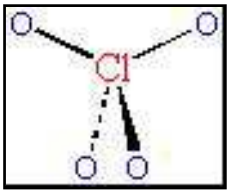
that the desirable properties of perchlorate and perchloric acid, combined with increased availability due to large scale production efforts, have led to uses in a wide variety of non-military applications and products. A partial list of these uses is provided in Table 1.

Table 1
Some Uses for Perchlorate Salts and Perchloric Acid (IME, 2004 & GFS, 2005)

Blasting agents	Brass and copper etching
Fireworks	Paints and enamels
Road flares	Leather tanning
Model rocket engines	Textile bleaching agent
Safety matches	Photographic flash powder
Automotive air bag initiators	Oxygen generators
Analytical testing agents	Ejection seats
Electroplating operations	Additive in polyvinyl chloride (PVC)
Electropolishing operations	Specialty industrial uses

This broadened industrial and commercial usage suggests the possibility that perchlorate contamination could be more widespread within Massachusetts than might be assumed.

2.2. Fate and Transport of the Perchlorate Ion



It is not only the expanded uses of perchlorate products that drive concern over accidental or incidental releases to the environment, but also its physical properties and mobility in environmental media, especially groundwater.

Specifically, perchloric acid and most perchlorate salts will readily dissolve in water, generating the perchlorate anion (ClO_4), a tetrahedral array of 4 oxygen atoms around a central chlorine atom. Although a strong oxidizing agent, the perchlorate anion is persistent in the environment, due to the high activation energy associated with its (abiotic) reduction to Chlorate (ClO_3). Moreover, given its relatively low charge density, perchlorate does not form complexes with metals in the same manner as other anions, and, in its ionic state, does not readily sorb to environmental media. [Urbansky, 2002] This combination of solubility, stability, and mobility creates the potential for both localized and area-wide impacts of toxicological interest.

2.3. Initial Detections of Perchlorate in Massachusetts

Perchlorate contamination of groundwater was first documented in Massachusetts in 2000 at the Massachusetts Military Reservation (MMR) on Cape Cod, as part of site assessment activities. A number of discrete plumes of perchlorate contamination have since been identified and characterized within the 15,000-acre Camp Edwards Impact Area and Training Ranges, emanating from a groundwater mound in the Northern portion of the base. Historical use of military munitions and flares are the suspected sources of contamination, which range from hundreds of µg/L in release areas, to single digit µg/L levels in the outlying edges of groundwater plumes. (http://www.mmr.org/)

In 2002, three municipal drinking water wells located just off the MMR boundary were found to be contaminated by low levels of perchlorate. The impacted community subsequently requested guidance from MassDEP on the health significance of this finding, which led to the issuance by the Department of a drinking water *Health Advisory* of 1 µg/L (see http://www.mass.gov/dep/brp/dws/percinfo.htm).

In the following two years, MassDEP continued to assess the toxicological significance of perchlorate, and began to obtain information that non-military releases of the contaminant were possible (e.g., via fireworks). In early 2004, the Department promulgated emergency regulations requiring public water supplies to test for perchlorate, as the first step in considering whether it was necessary and appropriate for the agency to promulgate a drinking water standard. As the data started to trickle in, discoveries of perchlorate in a drinking water source (groundwater or surface water) triggered field investigations designed to "back track" to the contaminant release area, and identify the source material(s). These efforts and experiences have led to an interim level of understanding of the nature and extent of perchlorate contamination across the state.

3.0 OCCURRENCE OF PERCHLORATE IN MASSACHUSETTS

The use, disposal, and/or accidental or incidental discharge of perchloric acid or perchlorate products could result in the contamination of environmental media, including surface water and groundwater. Recent reports have even suggested the possibility of the "natural" production of perchlorates in rain and in arid geological ecosystems. But how prevalent is perchlorate in Massachusetts, a region that is decidedly non-arid (44 inches of precipitation per year), and a state without a history of significant rocket propellant production or use?

Data from public water supply systems across the state provide a good starting point to begin answering this question.

There are approximately 450 community and 250 non-transient/non-community public water supply systems in Massachusetts, as plotted in Figure 1. The majority (89%) of these systems obtain water exclusively from groundwater aquifers. Collectively, this infrastructure constitutes a large, geographically and geologically diverse universe of water quality indicators.

Community public water supply wells in Massachusetts are comprised primarily of shallow overburden wells in water-table aquifers, providing a good vehicle to detect recent releases of soluble, mobile contaminants like perchlorate. Non-transient/non-community public water supplies in Massachusetts are comprised of extraction wells from both overburden and bedrock aquifers, servicing a variety of buildings and users (e.g., condominiums, schools).

In the last year, 85% (379) of the community and 86% (212) of the non-transient/non-community public water supplies in Massachusetts (groundwater and surface waters) have been tested for the presence of perchlorate, using analytical methodologies and laboratories capable of achieving a 1 μg/L Reporting Limit. *Of these 591 water supplies, only 12 sources in 9 water supply systems have detected perchlorate above 1 μg/L* (some systems have multiple groundwater production wells in close proximity). The communities where these 9 water supply systems are located are illustrated in Figure 2.

A summary of the relevant system parameters and findings for these 9 water supplies is provided in Table 2, including the range of perchlorate concentration values reported since the start of testing (early 2004).

As can be seen, perchlorate is not widely prevalent in public water supplies across the state, at least above 1 μg/L. Additional conclusions and observations of note in this regard are provided below:

- Although detections have been limited, they have occurred across the state, in a number of land-use and geologic settings, in both overburden and bedrock aquifers.

- The only impacted surface water supply was that for the Town of Tewksbury, which draws its drinking water from the Merrimack River, the state's second largest river, with a 5000 square mile watershed and average mean flowrate greater than 5000 cubic feet per second (CFS). In this case, the source of contamination in the river was eventually traced to an industrial user of Perchloric acid.

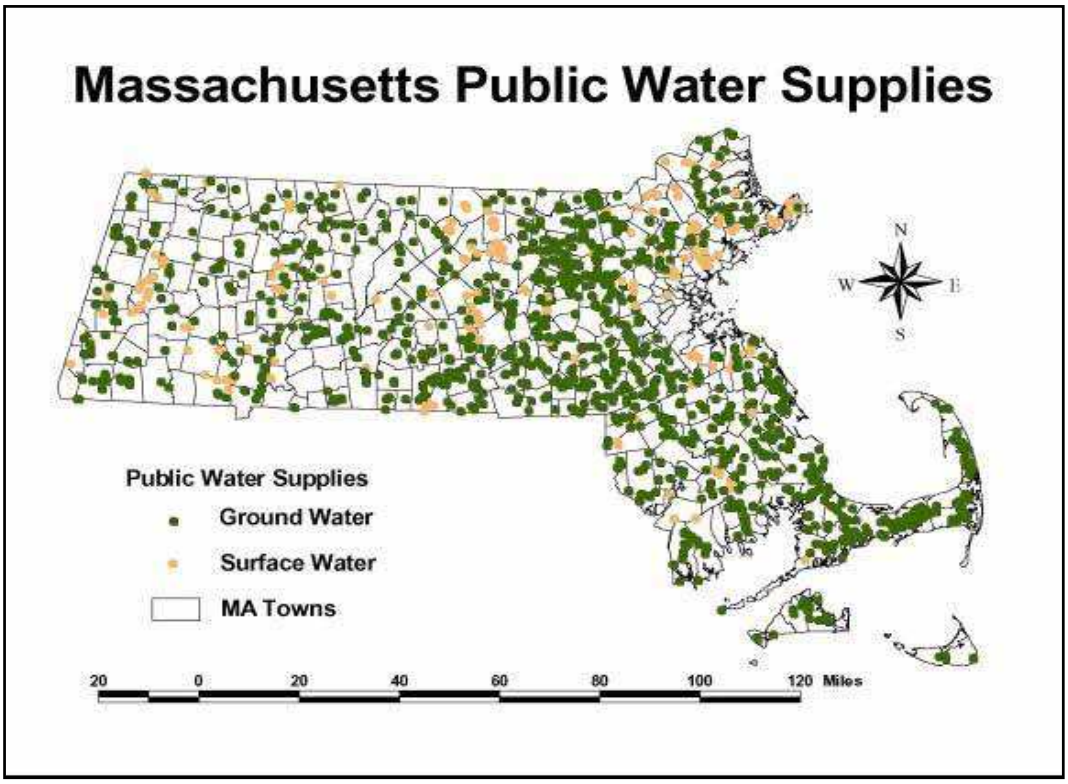


Figure 1 – Public Water Supplies in Massachusetts

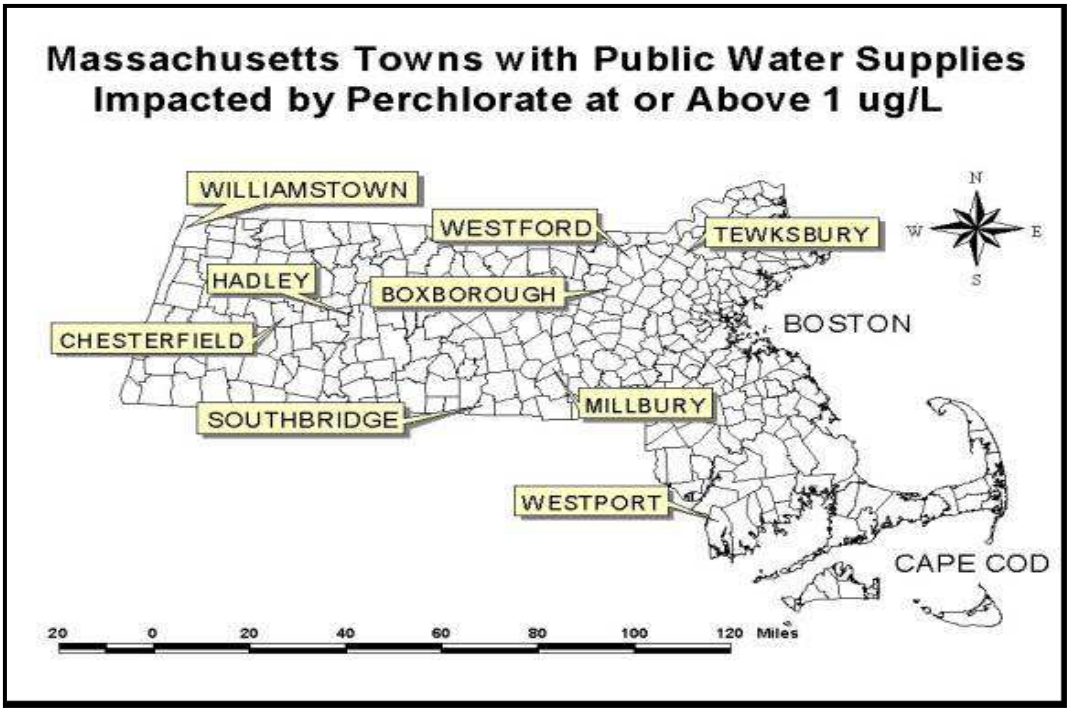


Figure 2 – Impacted Public Water Supplies in Massachusetts

Table 2
Massachusetts Public Water Supplies Impacted by at least 1 µg/L of Perchlorate
(Data current as of March 2005)

Town	System(s)	Description	Aquifer	Avg MGD	Sampling Rounds	Concentration Range µg/L	Likely Source(s)
Boxborough	Harvard Ridge	Condominium	bedrock	0.013	36	783 - 1300	Blasting
Chesterfield	Davenport Bldg	Town Office	bedrock	0.001	3	1-1.5	Fireworks
Hadley	Mt Warner Well # 2	Municipal water supply	overburden	0.720	6	1.5 – 3.8	Unknown
Millbury	Aquarian – Wells Jacques 1 & 2	Municipal water supply	overburden	1.664	8	16.1 – 45.3	Blasting
Southbridge	Indust Company Well # 1	Industrial Facility	bedrock	0.001	4	N.D. – 3.1	Unknown
Tewksbury	Merrimack River Intake	Municipal water supply	N/A	2.535	>50	N.D. – 3.26	Industrial Discharge
Westford	Nuttings Road	Municipal water supply	overburden	1.734	8	N.D. – 3.7	Blasting
Westport	High School 1 & 2	School	bedrock	0.001	13	1.06 – 3	Fireworks
Williamstown	Mt Greylock School 1 & 2	School	bedrock	0.005	14	1.03 - 10	Fireworks

- In 7 of the 9 cases, the source of contamination appears to have been identified, including: 3 situations where blasting activities occurred within one-half mile of the impacted water supply well(s), and have likely resulted in the observed perchlorate impacts; 3 sites where nearby fireworks displays appear to be the likely cause of contamination; and an industrial Perchloric acid user. The other 2 water supplies have shown low-level impacts up to 4 µg/L, without a clear source, although one system (Hadley) is located in an agricultural area where the use of perchlorate-containing fertilizers is possible.

One additional drinking water database is also available to provide some perspective in this matter: bottled water. Companies that sell bottled water in Massachusetts are regulated by the Massachusetts Department of Public Health (DPH), which establishes testing requirements for these products. Since early 2004, all bottled water purveyors have been required to test for the presence of perchlorate.

This testing information and data is available on the Massachusetts DPH web site at http://www.mass.gov/dph/fpp/pdf/perchlorate.pdf, and as of 12/7/04, contained test data for 50 bottled water products. These 50 products obtain their water from 7 locations in Massachusetts, 34 locations in 12 other states, 3 locations in Canada, and 6 locations in 4 other countries. *All of these products have reported perchlorate concentrations of Not Detected at a Reporting Limit of 1 µg/L.*

4.0 SOURCES OF PERCHLORATE IN MASSACHUSETTS

A number of reports exist documenting the nature and extent of perchlorate contamination at perchlorate production facilities, and at military installations, including the Massachusetts Military Reservation (MMR) on Cape Cod. However, despite our evolving knowledge on the use and/or presence of perchlorates in a wide variety of non-military products, little information exists on the "real world" impacts of these materials on surface and groundwater quality.

For this reason, the detection of the perchlorate ion in drinking water sources in Massachusetts triggered investigations by MassDEP to determine and examine the suspected source(s) of contamination. These investigations included site-specific assessment activities at and upgradient of the impacted water supplies, together with directed testing and evaluation programs of suspected source materials and activities.

On the basis of these efforts, in addition to military munitions, 3 other perchlorate-containing products in general commerce were identified as potential source materials of state-wide significance:

- Explosive Materials
- Fireworks
- Hypochlorite/Bleach Solutions

A fourth source of perchlorate contamination of a major water supply (Merrimack River) was found to be an industrial user of perchloric acid with a wastewater discharge to a Publicly-owned Treatment Works (POTW). While the prevalence of these types of users is unknown, it is clear that, on a mass-balance basis, such discharges can be a significant source of surface water and/or groundwater contamination.

4.1. Explosive Materials

Perchlorate salts (sodium, ammonium, and/or potassium) are used in some explosive materials, principally "water gels" and "emulsion" blasting agents, as well as some

blasting caps. Many questions remain, however, on where and how these products are used, and how they do or could impact environmental media, especially groundwater.

Water gels are explosive materials containing water, oxidizers, fuel, plus a cross-linking agent. Emulsions are explosive materials containing oxidizers that are dissolved in water droplets, surrounded by an immiscible fuel; or droplets of an immiscible fuel surrounded by water containing a dissolved oxidizer. Both types of products were first developed in the 1960s; presently, emulsions are more widely used than water gels. Both are sold and delivered in bulk form or as packaged products. (IME, 2004)

Most water gels and emulsions are classified as "blasting agents", as opposed to high explosives, because they are "insensitive" materials that are difficult to detonate. This is a beneficial attribute, for safety reasons. However, for certain difficult blasting applications, it is desirable to increase the sensitivity of these products; for example, at wet, water-saturated construction sites where the explosive is subjected to high static or dynamic pressures. Reportedly, perchlorate-sensitized blasting agents are among the best choices in these situations. (IME, 2004)

It is difficult to ascertain how much perchlorate is contained within a specific explosive material. This is because MSDS documentation provided for these products often specify a range, starting with zero percent, or a "less than" notation; for example:

- *Hydromite 400 Series* (Austin Powder Co)*:* 0-5% ammonium perchlorate and 0-5% sodium perchlorate (http://www.austinpowder.com/BlastersGuide
- *Dynosplit®E* (Dyno-Nobel): 0-15% sodium perchlorate http://www.dynonobel.com/dynonobelcom/en/global/
- *Slurran 915* (Slurry Explosive Corporation): <7% sodium perchlorate http://www.slurryexplosive.com/products.htm

During the course of MassDEP's investigation, the highest concentration of perchlorate encountered in an explosive material was "20% - 30%" for *Slurran XLS*, a watergel product manufactured by Slurry Explosive Corporation (SEC). While reportedly not added, small amounts of perchlorate (0.1%) could nevertheless be present in ANFO (Ammonium Nitrate/Fuel Oil), or other explosive products, given the use of Chilean nitrates by some manufacturers (e.g., see MSDS # 1019 for *Unimax*® by Dyno Nobel, at http://www.dynonobel.com/NR/rdonlyres/23F3B92C-2FCD-4475-9896-24D401BF88CD/0/1019PackagedDynandBlastingGel012405.pdf)

While the exact percentage of perchlorate salts in an explosive material may be difficult to obtain, the overall amount of this added chemical sensitizer is generally not

sufficient to change the material's status as a "blasting agent". This means that the product is still relatively safe, and will not detonate without a "boost" from other explosive charges. This in turn leads to the use of a series of explosive materials in and among blast holes, including detonators, primers, and boosters, loaded and fired in a manner and sequence to ensure the intra and inter blast hole "chain reaction" needed to detonate all elements in the explosive train.

The effective propagation and magnification of this shock wave - a transient pressure pulse that travels at supersonic velocity – is an essential prerequisite for ensuring the detonation of the perchlorate-containing explosive materials. A number of factors, however, can lead to one or more "misfires" in this sequence, including: an excessive gap between a primer or booster charge and the blasting agent, timing problems, formation characteristics, and, in the case of water gels, low temperatures.

4.1.1. Potential Environmental Release Mechanisms and Pathways

Perchlorate-containing explosive materials could result in environmental contamination and/or lead to human health exposures via the following activities, uses, and/or scenarios:

- *Misfires*. While misfires are a major industry concern and high priority – necessitating immediate and rigorous remedial efforts – it is not unreasonable to assume that some un-detonated product may not be recovered at some sites; especially if bulk or even packaged materials are scattered throughout a blasting zone as a result of the partial detonation of a blast hole. This could leave pockets of un-reacted perchlorate salts within the blast fragments/rock pile, and lead to the solubilization and mobilization of the perchlorate ion.

- *Placement (e.g., pumping) of bulk materials into open boreholes*. Depending upon the rheology and density of the agent, and the presence, degree and connectiveness of formation fractures, it would seem reasonable to speculate that some product could migrate out of a blast hole and not be detonated. This may be more of an issue for emulsion products, given that the cross-linking agent used in water gels leads to a reportedly stable gelatinous consistency.

- *Placement of compromised and/or opened packaged products into blast holes*. Packaged materials are often slit upon being loaded into a blasting hole, to allow them to more completely fill the full cross-sectional area, and/or to release any air within the packages and ensure sinking when lowered into wet holes. (IME, 2005). This again could place bulk/uncontained product into the open environment, with the concerns articulated above.

- *Bad Housekeeping.* Spills of packaged or bulk material to or into the ground, or insufficient misfire recovery efforts, can place or leave bulk/uncontained product in the open environment.

- *Blast Rock Processing*. Crushing rock blasted by perchlorate containing agents can generate dust and particulates that may contain trace levels of perchlorate (especially in the case of misfires). Run-off or washing operations of this rock can also result in surface water and/or groundwater pollution.

- *Normal Residuals.* The detonation of explosive materials is a violent chemical reaction, in which component molecules are thought to be instantaneously destroyed or decomposed by a pressure pulse moving through the material at supersonic speed. While it seems reasonable to assume that the residue from such a reaction should be essentially free of perchlorate salts, MassDEP has not to date seen industry data in this regard. Given the parts-per-billion concern with perchlorate in the environment, even "negligible" residuals from a large blasting effort may be of significance in this regard.

4.1.2. Blasting near Public Water Supply Systems

To date, MassDEP has obtained data from 3 sites in Massachusetts where blasting operations have resulted in the contamination of surface and/or groundwater with perchlorate, and apparent impacts to nearby drinking water wells. These sites are located in the towns of Millbury, Westford, and Boxborough. Available data on explosive materials used at each of these sites is provided in Table 3. All 3 locations employed the same blasting contractor.

4.1.2.1. Millbury

Blasting operations occurred at the Millbury site from July 10, 2002 through January 6, 2004. Much of the blast rock was reused at the site to facilitate construction of a large shopping mall, which was essentially constructed on the side of a bedrock hill (see Figure 3). Importantly, runoff from the roof drains of the mall buildings are discharged to the subsurface; in some cases into areas where blast-rock has been deposited.

In May 2004, perchlorate was detected in two (overburden) public water supply wells - Jacques # 1 & Jacques # 2 - at concentrations of 45.3 µg/L and 21.6 µg/L, respectively. Both wells were closed down, and MassDEP began an iterative search for the source(s) of perchlorate contamination, initially focusing on the

Table 3: Use of Explosive Products at 3 Construction Sites
(Per attestations of Blasting Company)

Town/ Dates	Explosives and Blasting Agents (Abridged List)				Perchlorate per MSDS?
	Product Name	Manufacturer	Type	Pounds	
Millbury 7/02 – 1/04	ANFO & ANFO WR	Dyno-Nobel	ANFO	621,252	Not Listed
	EZ-Det	Ensign-Bickford	Blast Cap	Not Avail	Not given
	Slurran 406	SEC	Watergel	74,257	Not Listed
	Detagel Presplit	SEC	Watergel	360	<7% SP
	Emgel ≥4 inches	MSI	Emulsion	2,332	Not Listed
	Emgel 2" & 3"	MSI	Emulsion	82,722	Not Listed
	Optiprime Boosters	Ensign-Bickford	Booster	Not Avail	Not Listed
Westford 8/03 - 8/04	ANFO & ANFO WR	Not Avail	ANFO	94,740	Not Avail
	EMGEL 200 & 250	MSI	Emulsion	474	Not Listed
	Hydromite 860	Austin	Emulsion	3,254	Not Listed
	Slurran XLS	SEC	Watergel	9,563	20-30% AP
	Slurran XG	SEC	Watergel	1,029	Not Avail
	Unimax	Dyno Nobel	Dynamite	5,088	Not Listed
Boxborough 11/03	Information not currently available				

sampling and analysis of nearby private drinking water wells, the Blackstone River, and contributing tributaries. By June, these efforts had traced contamination back to a mall development site located 1000 feet west of the impacted wells. By the beginning of July, confirmation was obtained that perchlorate-containing blasting agents were used at the mall development site.

The mall owners retained an environmental consulting firm, who proceeded to conduct additional investigative activities to identify the nature and extent of contamination – and look for other potential sources of perchlorate releases.

To date, assessment efforts have disclosed tens to hundreds of µg/L of perchlorate in surface water runoff systems, overburden monitoring wells, and bedrock monitoring wells on the mall property. In total, 9 private drinking water wells have been tested, though none appear to be directly downgradient of the mall area. None of these wells were found to contain perchlorate above 1 µg/L.

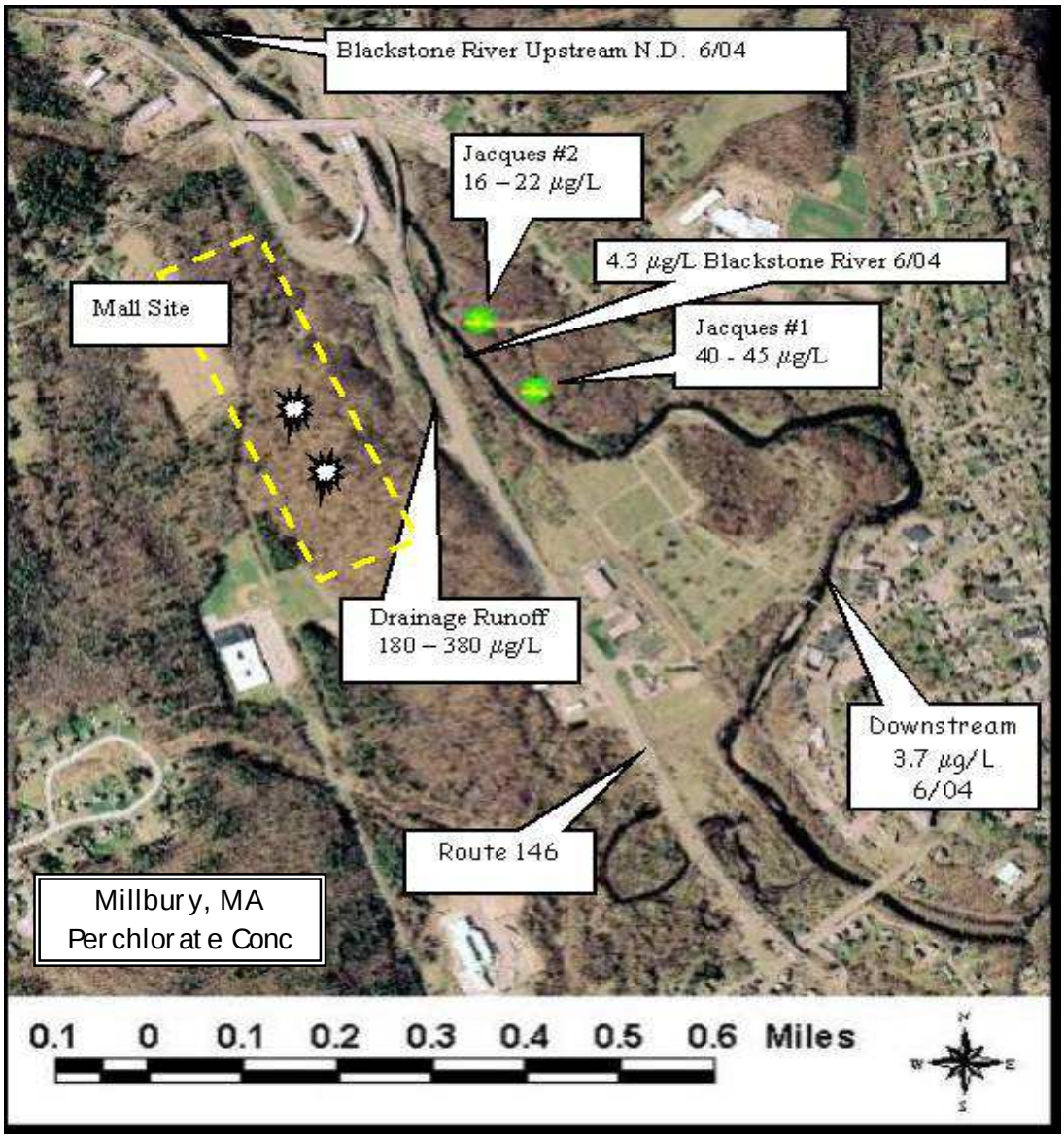


Figure 3: Millbury, MA Blasting Site

Monitoring wells upgradient of the mall site and upgradient of the presumed mall plume area have shown N.D. for perchlorate at a Reporting Limit of 1 µg/L. No other sources of perchlorate have been identified within the vicinity of this site.

4.1.2.2. Westford

Blasting operations occurred at the Westford site from August 26, 2003 to August 25, 2004, for the purpose of constructing a new municipal building (highway garage). The site is surrounded by a number of active and inactive

(rock) quarrying operations, which have presumably used a variety of explosive materials for decades.

In July 2004, 2 µg/L of perchlorate was detected in the Cote Well, a municipal water supply located approximately one-half mile northeast of the highway garage site (see Figure 4).

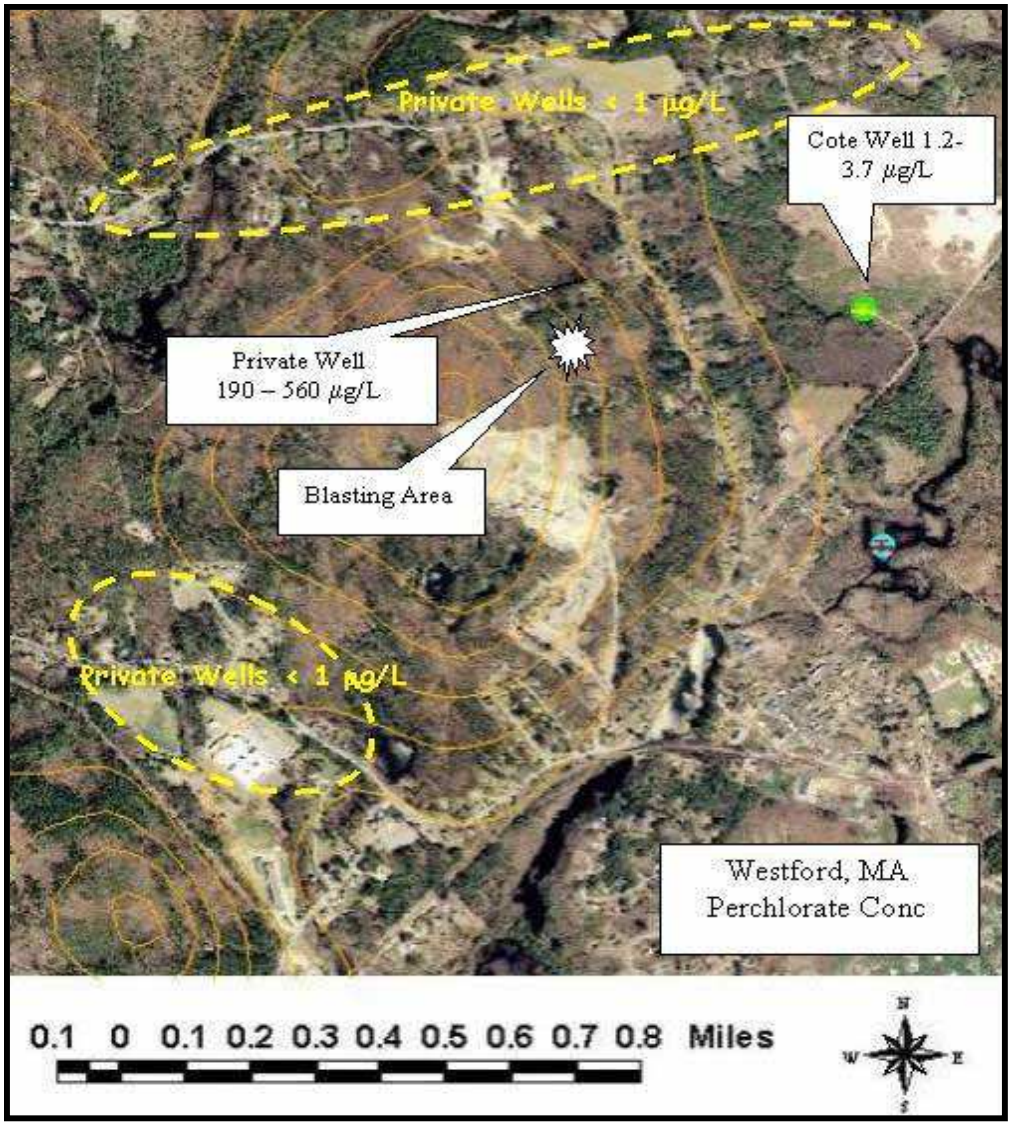


Figure 4: Westford, MA Blasting Site

This detection of perchlorate resulted in the shut down of the Cote well, and use of alternative water supply sources. It is interesting to note that two earlier rounds of sampling of this public water supply, in March and April 2004, reported N.D. for perchlorate at a Reporting Limit of 1 µg/L.

Following the shut down of the well, the Westford Water Department began to conduct additional testing of monitoring wells and surface waters. By early August, contamination was traced back to the highway garage location, via detections of tens to hundreds of μg/L of perchlorate in surface waters at and exiting the construction area. In mid-August, MassDEP began testing private water supply wells near the site. On August 23rd, data was received indicating the presence of 425 μg/L of perchlorate in a private drinking water well located within a few hundred feet of the construction site; the residents were immediately advised to cease using the water for drinking or cooking purposes. Over the next 4 months, 15 additional private drinking water wells within 4000 feet of the highway garage location were tested. Although these wells appeared to be hydraulically upgradient or cross-gradient from the suspected source area, some were drawing from the bedrock aquifer, and were sampled as a precautionary measure. All data from these wells were N.D. for perchlorate at a Reporting Limit of 1 μg/L.

Additional investigations were also conducted at an adjacent quarry, including sampling of on-site potable and process-water wells. Perchlorate was not identified, leading MassDEP to conclude that blasting at the Highway Garage site – using explosive materials that contained up to 30% ammonium perchlorate - appears to be the likely source of observed contamination.

4.1.2.3. Boxborough

Blasting was conducted at the Boxborough location during November of 2003, to facilitate the construction of a new wastewater treatment plant at a residential condominium complex.

In April 2004, 4.87 μg/L of perchlorate was detected in one of 5 on-site production wells. The other 4 wells reported N.D. In September, however, testing of a second well (Dunster House) identified 791 μg/L of perchlorate; a re-test two weeks later indicated 1080 μg/L. A peak concentration of 1300 μg/L was reported for this well in November 2004. (See Figure 5)

All five production wells are believed to be bedrock wells, spaced about 200 – 500 feet from each other. The most impacted well is located within several hundred feet of the blasting operations.

At the present time, MassDEP does not have information on the types and quantities of explosive materials used at this location, but suspects that perchlorate-containing blasting agents were among the inventory of products.

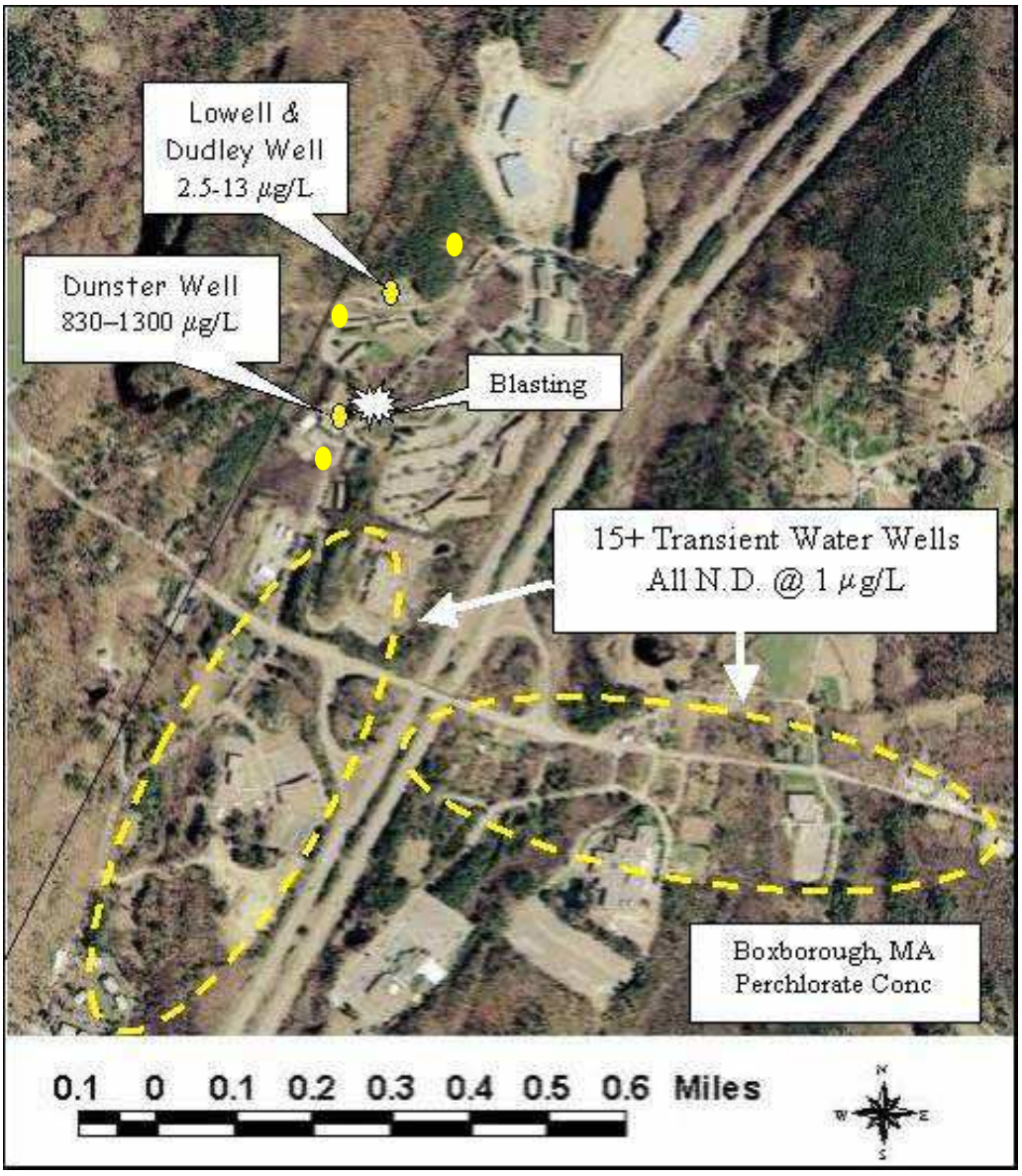


Figure 5: Boxborough, MA Blasting Site

In addition to the 5 condominium wells, approximately 20 other drinking water wells located within 1 mile of the site were sampled and analyzed for perchlorate, including 5 private wells and 15 "transient non community" public water supply wells. All results were N.D. at a Reporting Limit of 1 µg/L.

Because the condominium did not initially have an alternative water supply option, residents continued using the Dunster Well, until the end of 2004, though all were advised to use bottled water for drinking and cooking.

4.1.3. Discussion

The lines and weight of evidence appear sufficient to conclude that blasting activities at the 3 sites described above resulted in contamination of surface water and groundwater, and impacts to downgradient public drinking water supply wells:

- Perchlorate was present in blasting agents used at the Millbury and Westford sites, and is suspected at the Boxborough site;
- Environmental monitoring and assessment data are consistent with a source release within the area of blasting; and
- No other plausible sources or source areas of perchlorate contamination have been identified at any of these locations.

What is not clear is why contamination attributable to the use of explosive materials has only been observed at 3 public water supplies - out of a universe of almost 600 tested sources. Given the degree of construction (and blasting) activities in Massachusetts, and the environmental persistence and mobility of the perchlorate ion, why haven't more water supplies been impacted? Possible explanation include:

- Perchlorate-containing explosive products are relatively new formulations, and it would appear that their use has significantly increased in the last decade. It might take time for other impacts to be observed; and/or

- The specific practices and/or blasting agents used by the (same) blasting contractor at these 3 sites may have resulted in these (unintended and unanticipated) consequences.

Investigations and considerations in this matter continue.

4.1.4. Nitrate

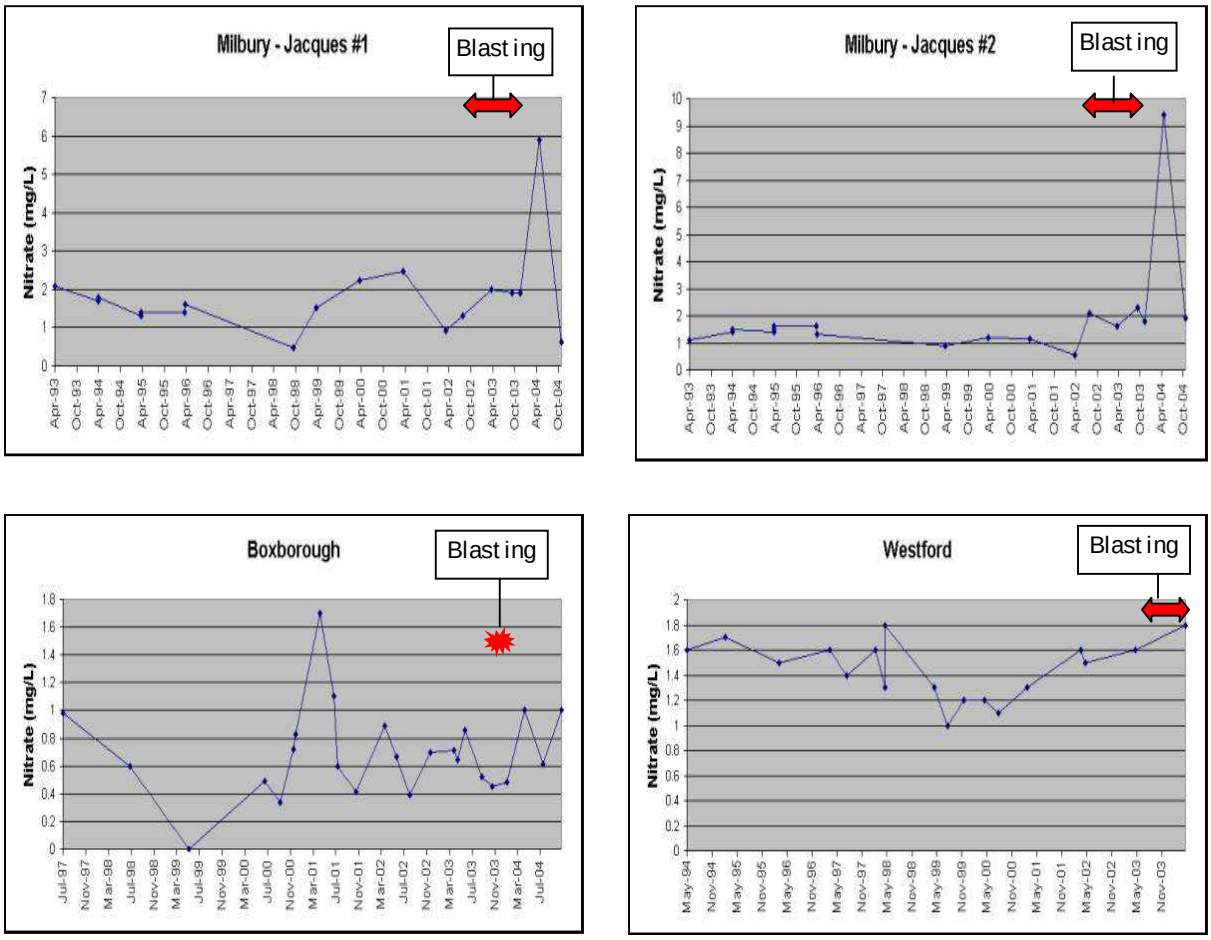
There is a blasting-related nexus between perchlorate and nitrate. Dissolved in an aqueous solution, both are anions, which result in significant groundwater mobility. Both are generally present in perchlorate-containing blasting agents. Moreover, perchlorate industry representatives have raised concerns over the potential environmental impacts from nitrates, which are by far the more predominant ingredient in explosives, including those products that would be used in lieu of perchlorate-containing blasting agents. For example, ANFO (ammonium nitrate + fuel oil) is commonly about 94% ammonium nitrate.

From a regulatory perspective, the 4-orders-of-magnitude disparity between the current nitrate drinking water standard of 10 mg/L and MassDEP perchlorate drinking water advisory of 1 µg/L suggests that an increased concern and emphasis on perchlorate is not unfounded. Moreover, MassDEP is not aware of any public water supply that became contaminated with more than 10 mg/L of nitrate as the likely result of nearby blasting activities.

However, there may be utility in establishing a perchlorate/nitrate link in blasting-related contaminated plumes, given that all water supplies routinely test for nitrates.

Figure 6 plots the last 10 years of routine nitrate monitoring data for the 3 blasting-related impacted water supplies.

Figure 6
Nitrate Levels in Wells Impacted by Perchlorate from Suspected Blasting Sources



The above data suggest the possibility of a relationship between nitrates and perchlorate at the Millbury site, given the 5-10 fold increase in nitrates in Jacques Wells # 1 and # 2, located 800 – 1000 feet to the east of the mall construction site, approximately 18 months after the start of blasting activities. This is also the site where large amounts of ANFO were used (621,000 pounds).

This relationship was further explored by the consulting firm overseeing work at the Millbury site, during a series of sampling events in February 2005, where split samples were analyzed for perchlorate and nitrates (NO_3-N). In total, 22 samples were synoptically analyzed in this manner, including 8 drainage/surface water samples, 8 overburden groundwater samples, and 6 bedrock groundwater samples. The results of all data are plotted in Figure 7. Once again, the possibility of a general correlation is suggested, though more evaluation of variables (e.g., site-wide explosive materials usage, precipitation events, groundwater elevations, etc.) would be needed to draw more definitive conclusions.

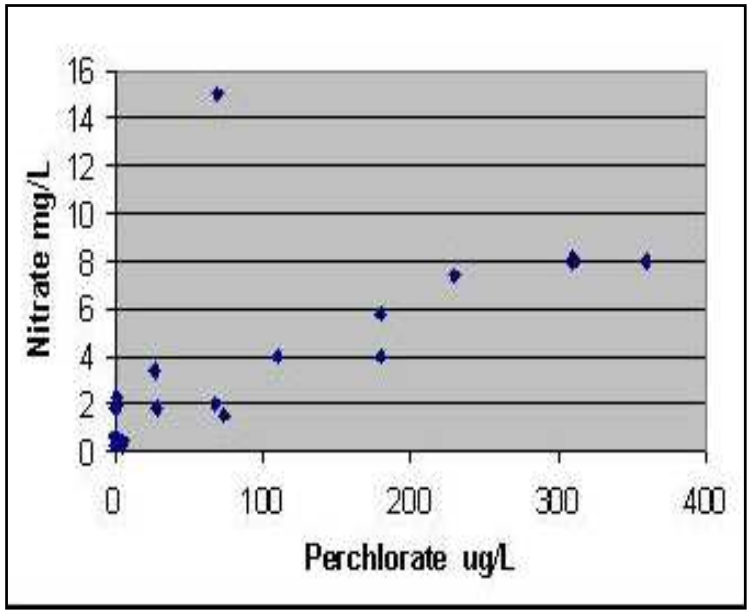


Figure 7
Millbury, MA Perchlorate vs Nitrate
(GeoSyntec, 2005)

A relationship between perchlorate and nitrate is not evident in the monitoring data for the Cote Well in Westford. This well is the most distant (2600 feet) and least impacted (3.7 µg/L) of the three blasting sites. Given these characteristics, and the fact that blasting did not begin until August 2003, it is possible that peak concentrations of both contaminants have not as yet been seen.

The lack of nitrate impacts to the Boxborough wells may be due to the formulation of the blasting agent(s) used for this construction project (not currently known). For example, Surran XLS, a perchlorate-containing watergel used in Westford, is comprised of (only) between 10 and 20% nitrates.

4.2. Fireworks

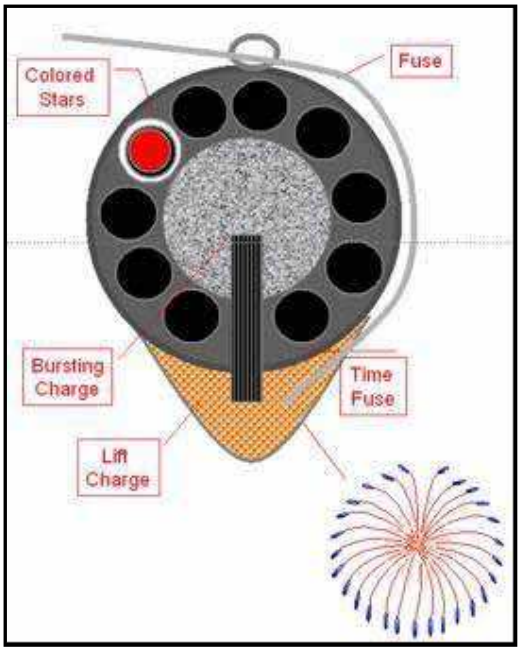
It has been difficult for MassDEP to obtain specific information on the chemical composition of fireworks.

By all accounts, most fireworks are manufactured in Asia (mainly China), using proprietary ingredients and formulations. Compositions are typically not listed on or provided for these products - just descriptive elements related to pyrotechnic colors, effects, and styles.

Industry sources have indicated two primary uses of perchlorates in fireworks:

- To produce color effects; and
- As flash powder in "Salute" shells (to produce a loud bang/flash).

Perchlorate use and content in fireworks has increased over the past two decades, in a (successful) effort to produce more vivid color effects (C&EN, 2001). Modern fireworks create these effects by the spectral emissions of excited gas-phase molecules, including barium chloride (green), strontium chloride (red), and copper chloride (blue). Potassium perchlorate is used as both an oxidizer as well as a chlorine donor in this process (bringing metal and chlorine together in a vapor state at high temperatures during the burning process). Perchlorate has replaced chlorate in this capacity for safety reasons; potassium salts are used (as opposed to sodium or potassium perchlorates) to limit interference with desired color emitters.



Fireworks color effects are most typically produced by the launching of aerial display shells, which contain numerous "stars" or small pellets containing a fuel/metal/oxidizer mixture. The frequency and extent of perchlorate use in these formulations – and whether those values are continuing to increase – is not clear.

In addition to color effects, potassium perchlorate is also used in a mixture with aluminum powder to create "flash powder". Containing up to 70% potassium

perchlorate, flash powder is used to create a loud noise and flash. Aerial shells containing flash powder are launched to provide "aerial salutes" during a display.

Aerial shells are packaged/wrapped in paper, and launched from a "mortar" (solid tube) using a black powder "lift charge". They range in size from 3 inches to 10 inches and more in diameter, and reportedly are launched 100 feet for every inch in diameter (http://pyrouniverse.com/professional.htm). There may be additional and expanding uses of perchlorate in the industry, given its availability, effectiveness, and relative stability and safety. Examples could include products available to the general public, including firecrackers and sparklers.

4.2.1. Potential Environmental Release Mechanisms and Pathways

Perchlorate-containing pyrotechnics could result in environmental contamination and/or lead to human health exposures via the following activities, uses, and/or scenarios:

- *Atmospheric Fallout*. Fine particles of burnt black powder, paper debris, and other chemical residues are the inevitable fallout from a fireworks event. The exact degree, nature, and extent of this fallout would seem to be highly site-specific, based upon the products used, weather conditions, and post-display cleanup (housekeeping) activities. This fallout could result in levels of perchlorate in soil, groundwater, and/or surface water. It could also result in inhalation exposures to perchlorate particulates during the display event.

- *Duds*. "Duds" are aerial shells that are launched from a mortar, fail to ignite in the atmosphere, and plummet back to the earth. Information available on the Internet suggests a common industry recommendation is to bury these shells for safety reasons. This could result in groundwater contamination from perchlorate salts within the shell.

- *Misfires*. Misfires are aerial shells that do not launch from the mortar. Information available on the Internet suggests a common industry recommendation is to apply water to/into the mortar for safety reasons. Uncontained run-off could result in soil and groundwater contamination from perchlorate salts within the shell.

While Massachusetts' regulations require collection and proper disposal of all debris, duds, and misfires, the degree of compliance is unknown.

4.2.2. Modeling of Potential Impacts from Fireworks Displays

MassDEP has conducted limited modeling efforts of hypothetical fireworks displays, in order to better define the scope and range of potential groundwater impacts and concerns. The details and results of this modeling effort are contained in Figure 8, which assumes a mid-sized "July 4th community display" of 1000 to 2000 aerial shells, with a total weight of 3000 pounds.

The average perchlorate content in all fireworks is assumed to be 40%, which is combusted in an aerial display, producing particulate/debris fallout that uniformly descends to the ground over a "football field" size area of 3600 square meters.

Beyond all of the normal areas of uncertainties in any generic analysis of site-specific events (e.g., wind speed and direction, atmospheric conditions and stability, hydrogeologic parameters), this analysis was further encumbered and limited by two key unknowns/variables:

- The amount of perchlorates used in fireworks, and
- The amount of perchlorates not consumed in the display (e.g., atmospheric fallout of un-combusted particulates and debris).

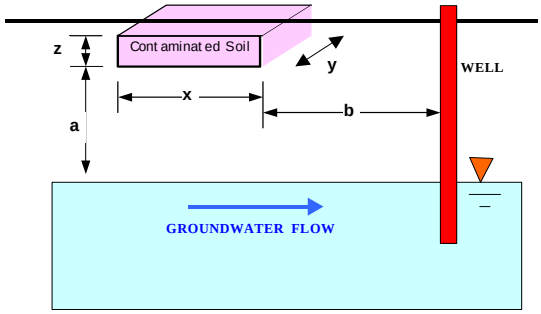
While the 40% perchlorate figure may be high, it is being used in the absence of anything more definitive from the pyrotechnics industry. On the basis of this analysis, even with 99.9% destruction of perchlorates, tens of μg/L of perchlorate could be expected immediately (100 meters) downgradient of the fallout area, with trace amounts (1 μg/L +/-) further downgradient. Higher concentrations could be expected with larger displays, use of pyrotechnics with higher amounts of perchlorates, less complete combustion, improper disposal of duds and misfires, excessive debris fallout and/or lack of post-display cleanup.

4.2.3. Fireworks Displays near Public Water Supplies in Massachusetts

Given the results of the generic modeling exercise discussed above, an effort was undertaken to geo-locate permitted fireworks displays with respect to proximate public water supplies.

In Massachusetts, the Office of the state Fire Marshall must permit all fireworks displays. In 2003, permits were issued for fireworks displays in 155 communities. Of these 155 displays, 47 were found to be located within the (calculated or assumed) groundwater recharge zones of public water supply wells (community and non-community water supplies). A total of 110 public drinking water supply wells

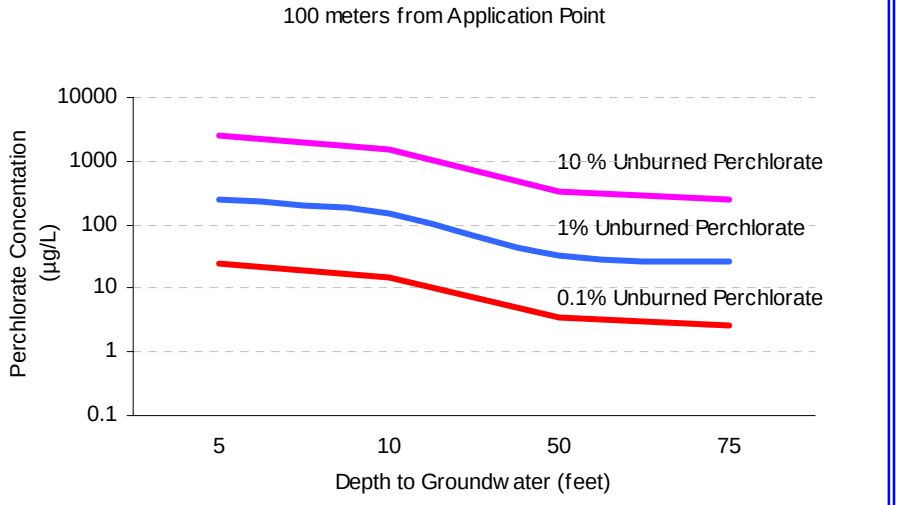
Figure 8: Modeled Perchlorate Impacts to Groundwater from Fireworks Display

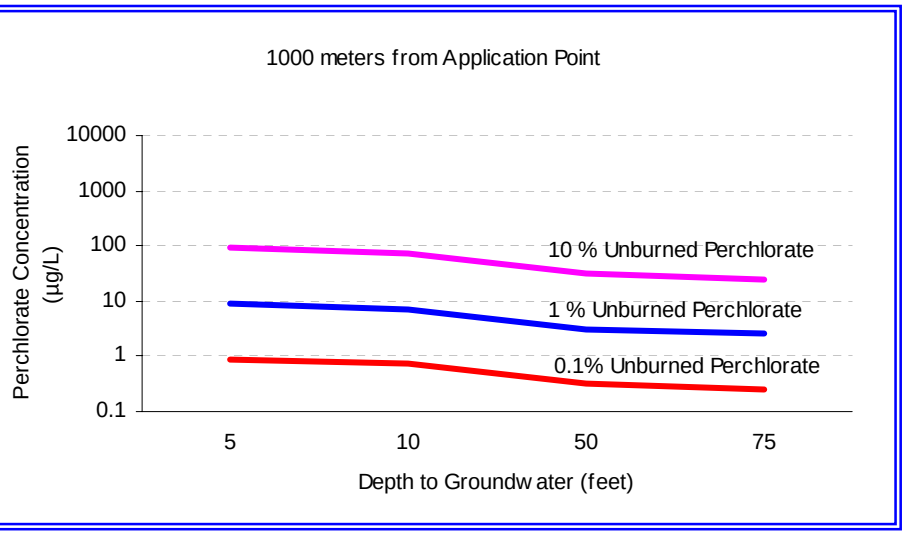


Model = SESOIL/AT123D

Assumptions:

- 3000 lbs fireworks (1000-2000 shells)
- Average perchlorate content = 40%
- x = 60 meters
- y = 60 meters
- z = 6 inches
- a = variable from 5 to 75 feet
- b = variable from 100 to 1000 meters





Additional Model Inputs:

- 44 inches of precipitation per year (Massachusetts)
- Solubility of Potassium Perchlorate = 1.5×10^7 µg/L
- Hydraulic Conductivity = 4.583 m/hr (sand)
- Hydraulic Gradient = 0.0031

are located within these 47 groundwater protection zones *(i.e., "Zone I/Is"* or *"Interim Wellhead Protection Areas"*). Of these 110 wells, 97 have been tested to date; all but one have reported N.D. for perchlorate at a Reporting Limit of 1 µg/L. One well, at the Mount Greylock School in Williamstown, has detected up to 10 µg/L of perchlorate.

This finding provides some comfort that fireworks displays have not resulted in the widespread contamination of public water supplies. While MassDEP has not as yet researched past records for fireworks events, most contemporary displays of major significance are held at the same location each year, so the 2003 data is believed to represent the majority of concern in this area.

Smaller and/or historical events will be investigated as contaminated public water supplies are identified. So far, MassDEP has determined that historic fireworks displays are the likely source of contamination in 2 of the 9 public water supply systems showing perchlorate levels above 1 µg/L: Chesterfield and Westport. These two supplies, along with the Williamstown School, are small, non-community wells drawing from bedrock aquifers. All three have low (primarily single-digit) levels of perchlorate; consistent with model predictions, as further detailed in Table 4, and discussed below in more detail.

Table 4: Public Water Supplies near Fireworks Displays

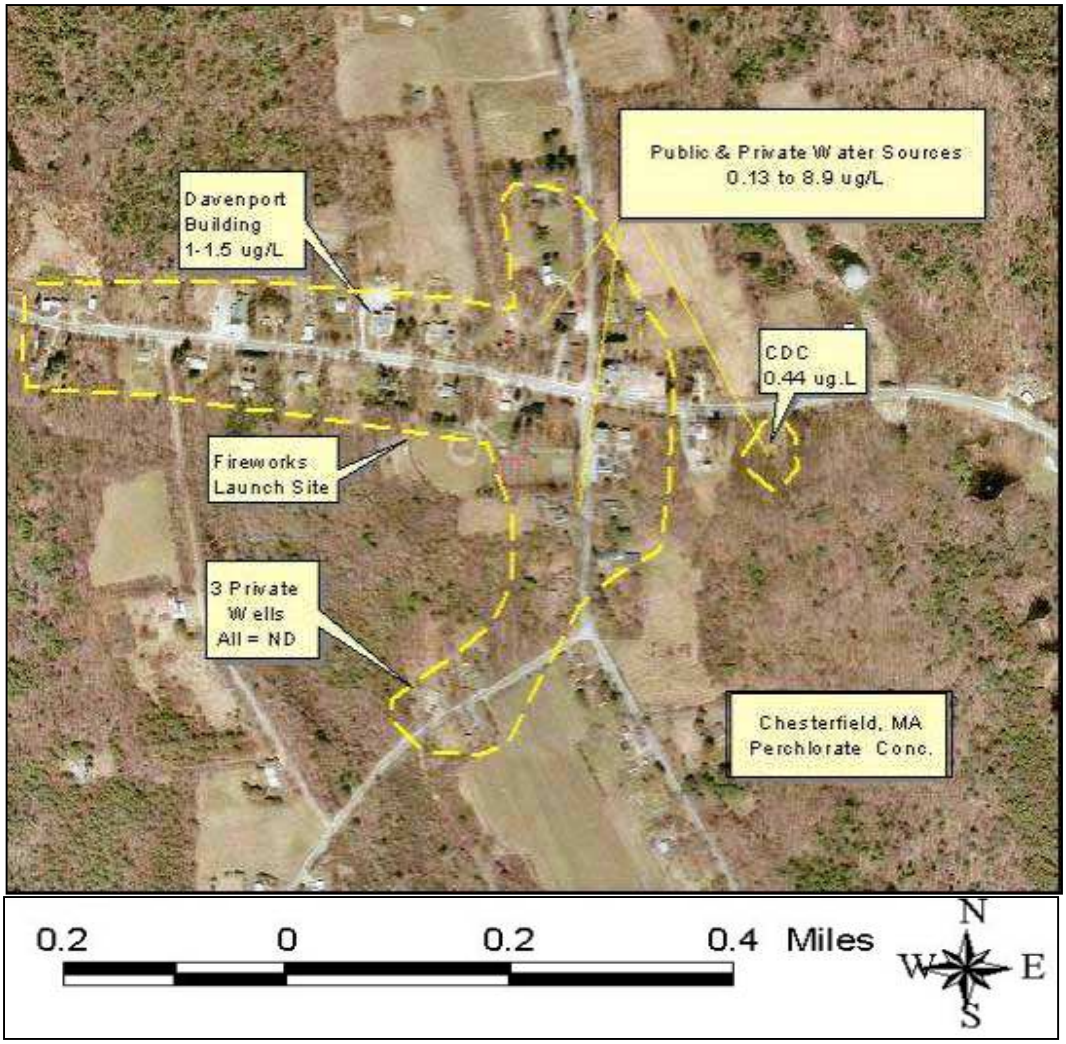
Town	Well(s)	Dist from Fireworks	Dates of Fireworks	Perchlorate Conc. (µg/L)
Chesterfield	Davenport Building	500 ft	Until 2002	1 – 1.51*
Westport	High School 1 & 2	600 ft	Mid 1990s	1.06 - 3
Williamstown	Regional School 1&2	800 ft	89-92; 99-03	1.03-10

* Nearby private well contamination up to 8.9 µg/L

4.2.3.1. Chesterfield

The Davenport Building is a small municipal facility in the Town of Chesterfield. On April 28, 2004, testing of the on-site well (considered a non-community/non transient public water supply) yielded 0.96 µg/L perchlorate. Follow-up testing in October and November 2004 reported 1.51 and 1.33 µg/L, respectively.

Although detailed records have not as yet been obtained, fireworks were reportedly launched from a municipal ball field located across the street from the Davenport Building, with the last event occurring on July 4, 2002 (see Figure 9).



Two residents from the area have recalled the existence of a significant amount of post-display debris; one resident stating that she had picked up five buckets of debris (5 gallons each) following one event. Recently, 29 private wells and two additional non-community public water supply wells within 1200 feet of the Davenport Building have been sampled and analyzed (via LC/MS/MS method). The data indicate detections of perchlorate in 17 of these wells, ranging from 0.13(J) to 8.9 µg/L, at a Reporting Limit of 0.20 µg/L. To date, no other confirmed or suspected sources of perchlorate containing materials have been identified at this location.

4.2.3.2. Westport

Fireworks were reportedly launched from the Westport High School for several years during the mid 1990s. On April 30, 2004, 3 µg/L of perchlorate was detected in the combined output from two bedrock production wells servicing

the High School, and located about 600 feet northeast of the former fireworks launch area (see Figure 10). Shortly thereafter, one well was taken out of service, and the remaining well has consistently reported perchlorate in the range of 1 to 2 µg/L.

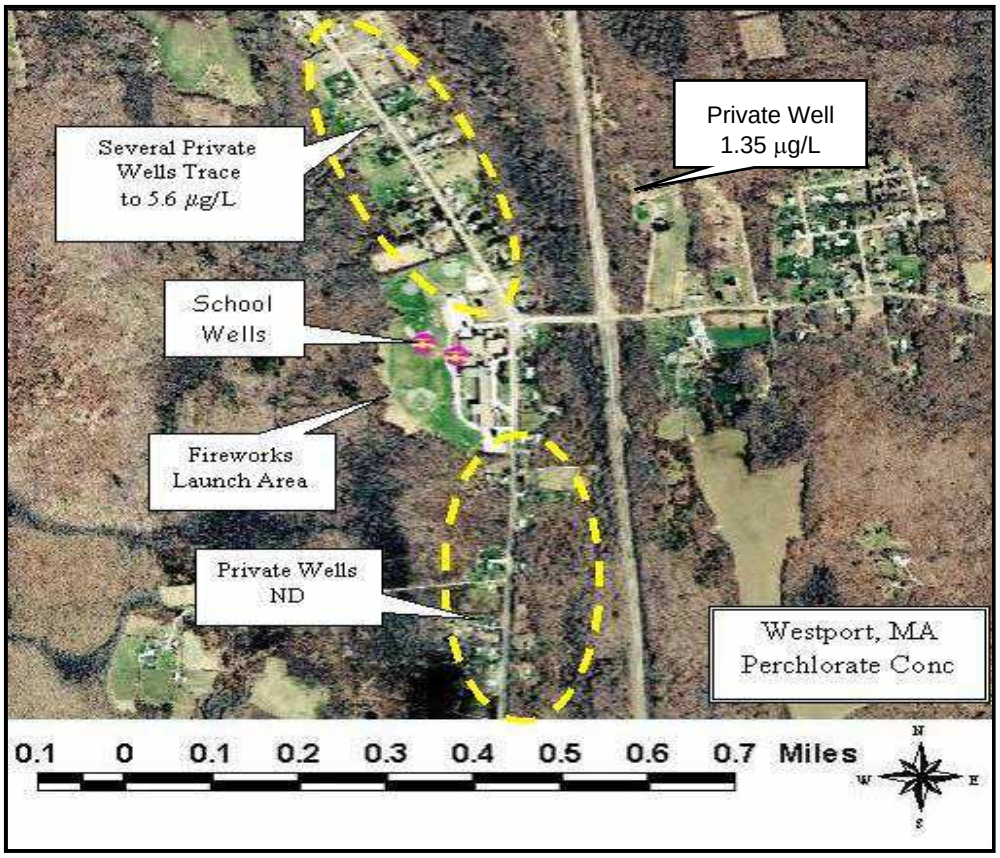


Figure 10: Westport, MA Fireworks Site

Groundwater movement in the area of the school is not known, but, based upon topography, is believed to be towards the south/southeast. Depth to groundwater is relatively shallow throughout the area (i.e., 10-15 feet below grade). The geology is expected to consist of glacial till overlying bedrock, with bedrock likely present 30 to 40 feet below grade. Importantly, the direction of wind during fireworks launching events is not known, though prevailing winds in this area are from the southwest.

This area of Westport is not serviced by a municipal water supply system, and homes surrounding the school obtain their potable water from on-property private water supply wells. In light of the detections at the school, MassDEP undertook a program to sample all wells within about a one-half mile radius of the fireworks launch area. In total, 30 private drinking water wells were sampled and analyzed via modified EPA Method 314; most homes were sampled

at least twice. Detections of perchlorates were reported in 8 of these homes, with 4 above the Reporting Limit of 1 µg/L. The maximum concentration was a value of 5.62 µg/L perchlorate in a home located about 1200 feet northeast from the fireworks launch area, and about 600 feet northeast of the impacted school wells. It is possible that other sources of perchlorate may be contributing to the low-level concentrations seen in these areas (e.g., hypochlorites).

One home with a point-of-use Reverse Osmosis filter system was sampled before and after treatment. In 3 rounds of synoptic sampling, the influent level of perchlorate fluctuated between 1.22 and 2.38 µg/L; the treated effluent was N.D. in all cases at a Reporting Limit of 1 µg/L.

4.2.3.3. Williamstown

Fireworks were launched from the Mount Greylock School in Williamstown between 1989 and 1992, and from 1999 to 2003. In April of 2004, two (bedrock) wells servicing the school were found to contain concentrations of perchlorate at 1.0 and 5.1 µg/L (see Figure 11).

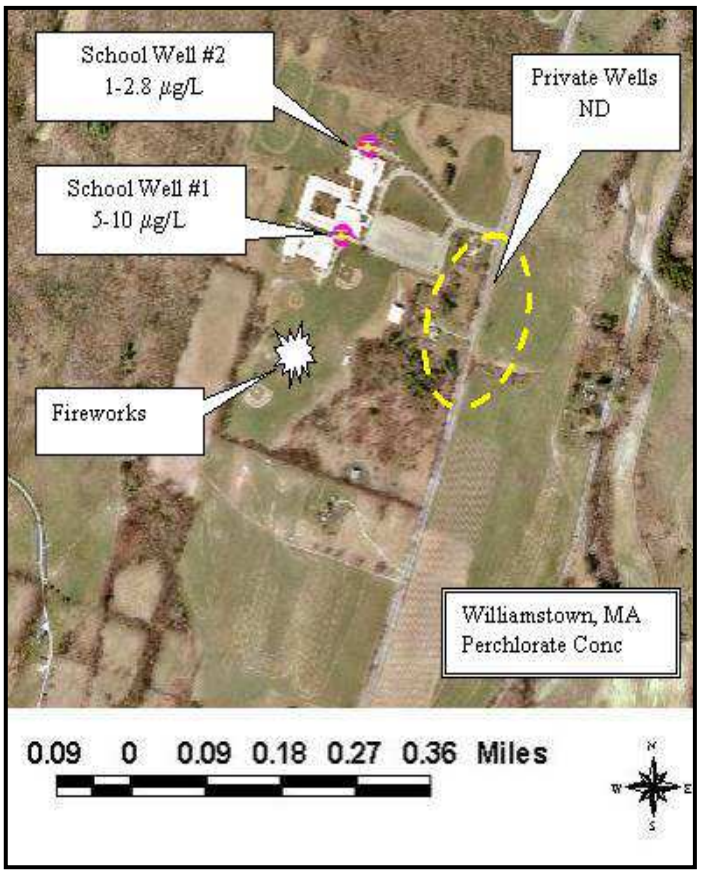


Figure 11: Williamstown, MA Fireworks Site

Two private wells located to the east of the school and within 1000 feet of the school and fireworks were ND at a Reporting Limit of 1 µg/L. The depths of these wells are not known.

Bedrock is believed to be present within 10 to 15 feet of the ground surface, and the groundwater table is believed to be in the bedrock. Investigations are continuing.

4.2.4. Bourne Fireworks Display

Between 1997 and 2004, fireworks were launched during July 4th celebrations at the Upper Cape Cod Regional Technical School in Bourne. This launch area is located approximately 700 feet westerly of the Massachusetts Military Reservation, and 400 feet southwest of a groundwater contaminant plume containing explosive constituents, including perchlorate. One of 4 major perchlorate contamination areas under study at the 15,000-acre military installation, this 4500-foot, 318 acre plume contains predominantly single-digit concentrations of perchlorate, flowing in a northwest direction towards the Cape Cod Canal. The highest concentration of perchlorate in the plume is approximately 19 μg/L (see Figure 12), as opposed to higher perchlorate levels (several hundred μg/L) in other areas of the base.

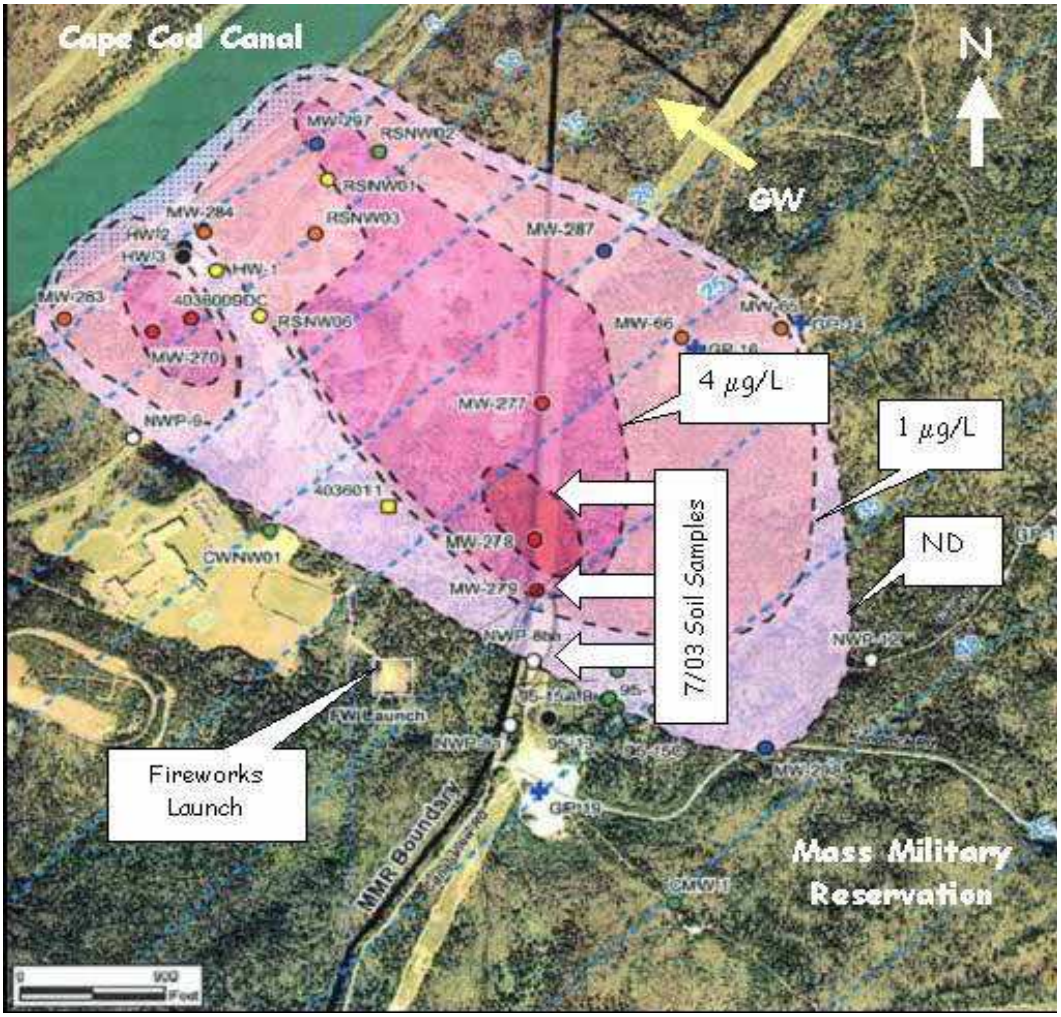


Figure 12: Bourne, MA Fireworks Site

In July 2003, a military contractor collected soil samples along the western border of the base before and after the annual July 4th fireworks display at the Technical School. At 3 locations 1000 – 2000 feet northwest and downwind from the launch site, in an area containing fireworks paper debris, post-event surficial soil samples were found to contain 1330, 1260, and 7560 μg/kg of perchlorate, compared to a pre-fireworks level of N.D. Two of these locations were re-sampled 2 months later, on 9/18/03 and 9/23/03, and were found to have gone from 1330 μg/kg to 5.3 μg/kg, and from 7560 μg/kg to 15 μg/kg perchlorate. The fireworks paper debris was also analyzed, and found to contain between 302 and 34,200 μg/kg of perchlorate. (AMEC, 2004)

It should be noted that to date MassDEP has not concluded that fireworks launched from the Technical School are the primary source of perchlorate identified in this "Northwest Plume". Contrary considerations in this regard are the known use of perchlorate-containing materials on the military base, and the presence of perchlorate 30 to 40 feet into the surficial water table in the downwind/ deposition area of concern (i.e., not clear why perchlorate ion would flow in a downward vertical direction to this depth in this presumed source area). Nevertheless, this investigation and data indicate that (a) measurable concentrations of perchlorate can be found in surficial soil thousands of feet downwind of a fireworks launch area, (b) perchlorate is not "completely combusted" in aerial display shells, and (c) debris fallout may be the most significant fireworks-to-surficial-soil mass-transfer mechanism.

4.2.5. Easthampton Fireworks Display

For a number of years, a July 4th, community-type fireworks display event has occurred at Galbraith Field in Easthampton. Located off Taft Avenue, Galbraith Field is a multi-acre athletic facility owned by the Williston Northampton School. It is underlain by an extensive system of sub-drains, presumably installed some years ago to dewater the fields by depressing the groundwater table and/or intercepting infiltrating rainwater and snowmelt. These sub-drains connect to a network of catch basins and outfalls which discharge into a wetland area adjacent to White Brook, which then flows in an easterly direction into Nashawannuck Pond.

A limited sampling effort was undertaken in November 2005, involving the collection and analysis of 8 soil samples, 2 sediment samples, and 8 water samples for perchlorate. As a result of this effort, perchlorate was not identified in any soil or sediment sample, at an analytical reporting limit of approximately 50 μg/kg. However, perchlorate was detected in 5 water samples, with the highest value of 6.62 μg/L identified in an outfall of the sub-drain system that discharges to a

wetland southeast of the field. This finding is consistent with modeling projections and data from other sites, with respect to "None Detect" concentrations of perchlorate in both soil and sediment samples, and 10 μg/L to 100 μg/L concentrations of perchlorate within the groundwater underlying the launch and fallout areas (given the expected dilution within the sub-drain system from non-impacted areas).

Of additional interest in the Easthampton study is a finding of low-levels of perchlorate (approximately 0.2 μg/L) in White Brook upstream of areas likely impacted by the Galbraith Field fireworks events. This suggests an area-wide "background" level of perchlorate due to unknown sources in higher reaches of the watershed.

4.2.6. Dartmouth Fireworks Study Area

The University of Massachusetts at Dartmouth has hosted one or more community fireworks displays in 9 of the last 10 years. In this time period, 11 events have occurred. Weather data obtained by MassDEP from 1996 to the present documents the prevailing wind direction on the date and at the time of fireworks launching to be predominantly to the north/northeast (70% of events). This is consistent with observations and statements made by campus officials.

In the Spring of 2004, MassDEP was granted permission by the University to install groundwater monitoring wells in and around the fireworks launch area, in an attempt to better understand groundwater impacts from suspected perchlorate-containing pyrotechnics. In total, 8 groundwater-monitoring wells were installed by MassDEP in June and August of 2004, including 4 small-diameter "direct push" wellpoints, and 4 additional 2-inch diameter wells installed via hollow-stem auger techniques. All wells were screened at the water table interface, which was about 5 feet below grade across the study area. Soil conditions in the area consisted of glacial till with large cobbles and small boulders. Bedrock is believed to be 20 to 30 feet below grade within the study area.

A fireworks event occurred on the campus on September 6, 2004, under calm wind conditions. According to records provided to the local fire department, the fireworks program consisted of a total of 1,750 aerial shells.

Prior to the September 6th event, surficial (0-1 inch) soil samples had been obtained and analyzed from the launch area, along with groundwater samples from the 8 monitoring wells. On the morning of September 7th, following a clear night without rainfall, soil samples were again collected from the same pre-event locations. One week after the fireworks display, following the first significant rainfall event,

groundwater samples were obtained from all 8 monitoring wells. Additional rounds of groundwater samples were obtained in October and December of 2004, and February of 2005. The location of key site features and monitoring points, along with all groundwater data, is provided in Figure 13.

As can be seen, fireworks were launched in a 500 foot by 300 foot field southwest of the campus center. Surficial soil samples obtained in this area prior to the launch (June 2004) were all N.D. for perchlorate. Surficial soil samples obtained in this area on September 7th ranged from N.D. to 560 µg/kg perchlorate.

Groundwater data for the 8 monitoring wells over all sampling rounds ranged from N.D. to a high of 62.2 µg/L of perchlorate. Concentrations have slowly declined over time in the 5 wells nearest the launch area. However, there has been no discernable "spike" in groundwater concentrations post September 6th; in fact, the high concentration of 62.2 µg/L perchlorate was recorded in August 2004 - prior to the latest display. Moreover, some of the highest levels of perchlorate are seen in wells UMD-7, 3, and 2, which are hydrologically cross and/or up gradient from the primary launching (mortar) sites.

Further analysis of site information and data suggest possible explanations for these observations:

- A likely (and perhaps most significant) pathway for perchlorate introduction to the groundwater from fireworks events is via fallout of aerial debris (e.g., pieces of un-combusted aerial shells). The predominant wind direction at this site is to the north/northeast, counter to the direction of groundwater flow. This could explain the elevated perchlorate concentration in the upgradient wells: the remnants of 10 years of fallout and surficial deposition.

- Based upon slug testing of wells UMD-5, 6, and 7, and consistent with the observed and expected geologic conditions, the hydraulic conductivity of site soils (at the water table interface) was calculated to be in the range of 10^{-3} to 10^{-4} cm/sec. Given the average hydraulic gradient across the site of 0.0167 ft/ft, groundwater velocity is expected to be in the range of 0.04 to 0.4 ft/day, or about 15 to 150 feet per year. This means that groundwater is moving relatively slowly, and would explain why the heart of the perchlorate plume has not yet moved beyond the launch area (i.e., still moving downgradient from the up-wind deposition areas).

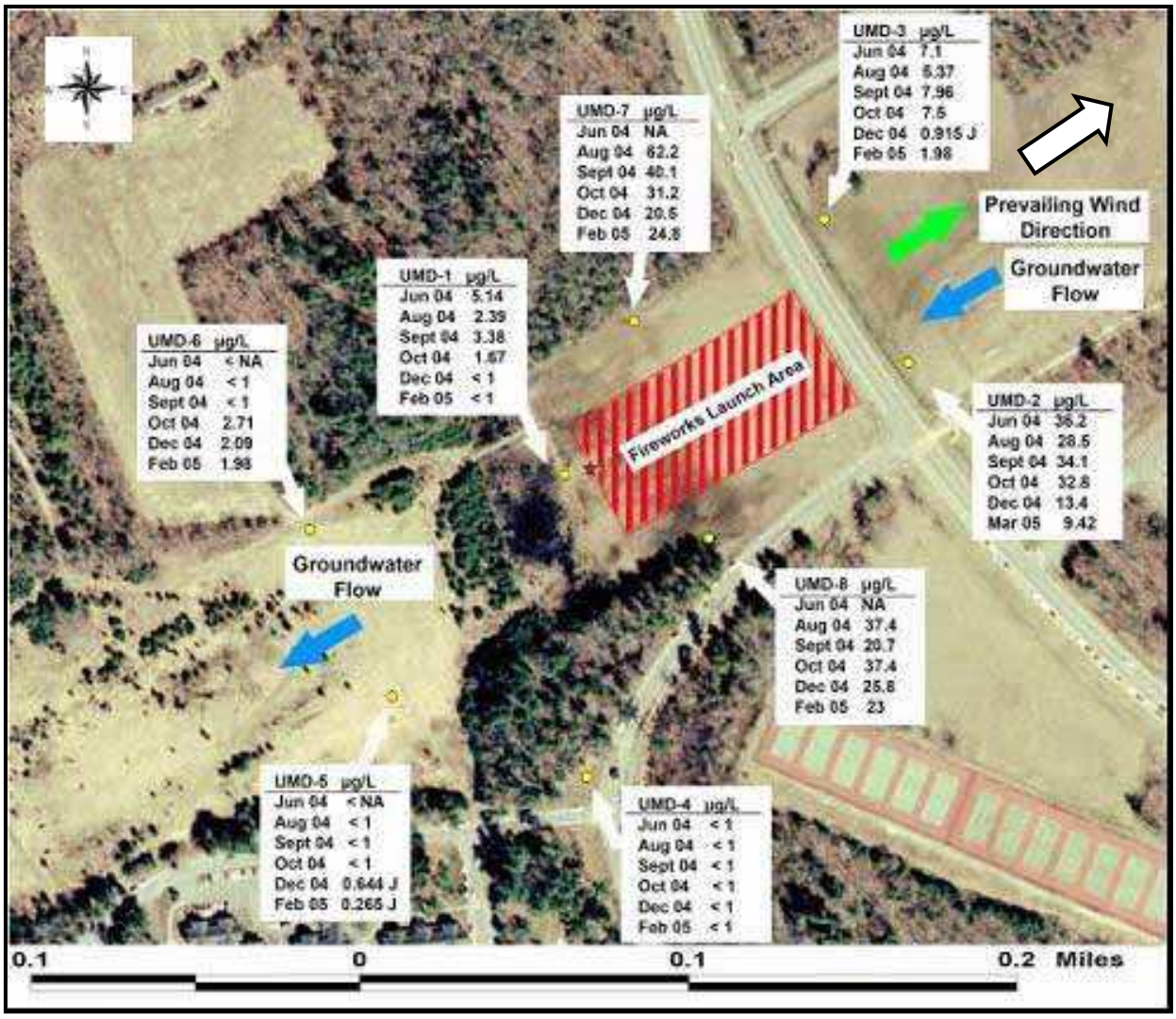


Figure 13
Fireworks Study Area, University of Massachusetts at Dartmouth

Other potential sources of perchlorate were investigated at this location, and are not likely to be a factor in this evaluation:

- While blasting activities have occurred at and proximate to the University, the nearest location is more than 2000 feet from the fireworks study area, in a likely cross-gradient groundwater direction. Moreover, available records do not indicate the use of perchlorate-containing explosive materials, or even water gels or emulsion explosive materials, which are the most likely to contain perchlorate salts.

- According to campus officials, herbicide use is limited in this area, and there is no reason to believe that chlorate-containing products have or would have been used (since these may contain perchlorate salts as impurities).
- While the use of Chilean fertilizers is always a (remote) possibility, it does not seem likely.
- Finally, the fireworks study area is located on the side of a small hill. If the groundwater table mirrors the surface topography, which is the expectation in geologic settings of this nature, the area of upgradient groundwater recharge is limited to only about 20 – 25 acres, in the predominant down-wind direction, on land containing (30 year old) university buildings and open spaces.

Additional information and data is available on the investigations at the Dartmouth campus at http://www.mass.gov/dep/brp/dws/percinfo.htm

4.3. Hypochlorite/Bleach Products

In the course of investigating the source of perchlorate contamination to the Tewksbury public water supply, data was obtained indicating the presence of perchlorate in hypochlorite disinfecting solutions. This has led MassDEP to conduct additional research in this area, to better define the scale of potential impacts from these materials.

4.3.1. Chemistry of Hypochlorite Products

The most common type of hypochlorite/bleach solution is sodium hypochlorite, NaOCl, a greenish-yellow liquid solution. A lesser-used salt is calcium hypochlorite, a white powder that is often used for swimming pool chlorination.

The primary method of manufacturing sodium hypochlorite is by reacting a dilute solution of caustic soda (NaOH) with liquid or gaseous chlorine. The end product is then processed and mixed to user specification. Typically, the concentration of sodium hypochlorite in commercial products range from about 6% (by weight) in household bleach, to up to about 16% (by weight) in products delivered and used at water and wastewater treatment facilities. (Powell, 2002)

Sodium Hypochlorite solutions are not stable, and "decomposition" is a well-known industry problem and concern. The most prominent degradation pathway results in the production of chlorate:

$$3OCl^- \longrightarrow ClO_3^- + 2Cl^-$$

In a basic solution, decomposition has been shown to be a second order process, i.e., Rate = k_2 $[OCl^-]^2$. (Gordon, 1996) Manufacturing specification typically set a limit of 1500 mg/L (ppm) of chlorate in delivered products. (Powell, 2002)

Steps can be taken in the manufacturing and post-production phases to minimize breakdown of the hypochlorite ion, by adding excess caustic soda to maintain a high (>11) pH condition. In addition, filtering is typically undertaken by manufacturers to remove transition metals (e.g., nickel, copper) that might have been present in the caustic soda feed stock. (Powell, 2002) These metals are known to catalyze a reaction that converts the NaOCl to O_2 (oxygen), lessening the (disinfecting) strength of the product, and potentially creating operational and safety problems:

$$2NaOCl \longrightarrow 2NaCl + O_2$$

Ionic strength and temperature are also key factors in controlling product breakdown during storage. Diluted product will degrade at a slower rate. Cooler storage temperatures also helps: one equipment manufacturer has indicated that for every 10°C increase in storage temperature, degradation of hypochlorite to chlorate will occur at a 3.5 times faster rate. (Powell, 2002)

Differences in manufacturing processes, quality control, and storage conditions will lead to differences in product chemistry. According to industry literature, it is clear that sodium hypochlorite solutions can become "enriched" in chlorate over time. *Moreover, based upon limited data obtained by MassDEP during this study, it appears that the chlorate may in turn break down over time into end products that include perchlorate.*

The chlorate-to-perchlorate pathway is well established. At present, the commercial production of perchlorates relies almost exclusively on the electrochemical conversion of chlorates. Other (less efficient) pathways are also known to exist, including 2 mechanisms of potential relevance to hypochlorite solutions:

- *Thermal Decomposition of Chlorates* – Through a "self-oxidation" process, chlorate salts have been shown to decompose to perchlorates (Schumacher, 1960). For example, in the case of sodium chlorate:

$$4NaClO_3 \longrightarrow 3NaClO_4 + NaCl$$

This approach is not considered commercially viable, however, because of energy and material requirements, as well as inherent difficulties in maintaining optimum production conditions, including production irregularities due to the "catalytic effect of impurities". (Schumacher, 1960) While significant production of perchlorates in this manner can only occur at high temperatures, it seems reasonable to speculate that "parts per billion" levels of perchlorate production could occur at room temperature over an extended period of time.

- *Chemical Oxidation of Chlorates* – The reaction of strong oxidizing agents with chlorates, including ozone, is known to result in the generation of perchlorates. (Schumacher, 1960). This leads to speculation over possible interactions between the (major) hypochlorite decomposition pathway that produces chlorate and the (minor) hypochlorite decomposition process that produces O_2; are intermediate by-products and/or related reactions oxidizing (a small percentage) of chlorate to perchlorate?

4.3.2. Perchlorate in Commercial Hypochlorite Products

During the agency's investigation of wastewater discharges to the Merrimack River – the source of the Tewksbury water supply – samples of sodium hypochlorite solutions were taken from the City of Lowell and Town of Billerica Wastewater Treatment plants, for analysis for perchlorate by EPA Method 314. When this data indicated positive detections, MassDEP sampled hypochlorite solutions at the Lowell and Billerica wastewater plants – together with a sample of the hypochlorite solution used at the Tewksbury water treatment plant, for analysis for perchlorate by both EPA Method 314 and an LC/MS/MS technique (EPA Method 331.0, available at http://www.epa.gov/safewater/methods/met331_0.pdf).

This data is provided in Table 5.

These data provide (a) empirical proof of the presence of perchlorates in the hypochlorite solutions; (b) evidence of potential differences in product chemistry among suppliers/manufacturers, and (c) indications of a relatively good correlation between the EPA 314 method and LC/MS/MS technique.

On the basis of the above findings, the Town of Tewksbury conducted an additional evaluation of a newly received shipment of product, as detailed in Table 6.

Table 5: Sampling of Commercial Hypochlorite solutions
October 8, 2004

Plant	Percent Hypochlorite	Manufacturer	Perchlorate Conc (µg/L)	
			EPA 314	LC/MS/MS
Lowell WWTP	NaOCl - 15 %	Univar	1500J	3400
	NaOCL - 15%	Jones Chemical	<900	260
Billerica WWTP	NaOCl - 15%	Univar	4100J	4600
Tewksbury WTP	NaOCL - 15 %	Univar	3000J	4100

Table 6: Hypochlorite Study by Town of Tewksbury Water Treatment Plant
(Ladderbush, Zediana, 2004)

Hypochlorite Solution (Univar 15% NaOCl)		Perchlorate µg/L (LC/MS/MS)
Bottom of tank before delivery		4380
New Delivery		<0.2
Aged 26 days	Stored in Dark @5 C, capped	995
	Stored in Dark @5 C, capped	1020
	Filtered (DE), Stored in Dark @ 5 C, capped	490
	Stored in Dark @ Room Temperature, capped	6750
	Stored exposed to air & light, Room Temperature	3050

Data from the Tewksbury study are consistent with the expectations on the breakdown of NaOCl to chlorate, in that perchlorate concentrations are "enriched" with increasing storage times. Similar to chlorate, lowered temperatures significantly lessened perchlorate production. Although chlorate concentrations

were not obtained during this study, these findings do suggest a possible correlation between chlorate and perchlorate production in hypochlorite solutions.

The filtering of the newly delivered hypochlorite solution by DE (diatomaceous earth) is interesting, with respect to the substantially reduced levels of perchlorate at day 26; is something being removed that is facilitating or catalyzing a reaction? Diatomaceous earth is used to filter freshly manufactured hypochlorite solutions, to remove metal impurities that are known to catalyze reactions that convert NaOCl to O_2. (Powell, 2002) The DE used by the Town of Tewksbury in this experiment was EaglePicher Celatom® FW-14, a product used in their water filtration plant. Did this filtering operation remove transition metals, lessening decompositional generation of oxygen, which lessened the conversion of chlorates to perchlorates; and/or perhaps removed other "impurities" that were mentioned by Schumacher in his discussion of the "self oxidation" reactions involving chlorate?

4.3.3. Perchlorate in Household Bleach

Given the occurrence of perchlorate in commercial hypochlorite solutions, MassDEP conducted a limited investigation of household bleach products in December of 2004. Specifically, 4 bottles of products were obtained from local supermarkets. An attempt was (successfully) made to find an old product, to investigate the "aging" concern. All samples were promptly analyzed for perchlorate content by LC/MS/MS techniques. The data is provided in Table 7.

Table 7: Perchlorate Content of 4 Household Bleach Products

Brand	Brand Info	Perchlorate µg/L
Clorox Ultra Regular 1.5 pint size	6% NaOCl Made in USA	370/320 (blind duplicate samples)
Shaws Ultra Bleach 1.5 qt size	No NaOCl content given Made in Canada	8000
Market Basket Ultra 1.5 qt size	6% NaOCl (no info on where made)	390
Wal-Mart Ultra Bleach 3 qt size	6% NaOCl by wt Made in Canada	89

Of note is the 8000 µg/L value listed for the Shaws Ultra Bleach. According to the markings on the bottle (which were specifically sought out), this product was

manufactured 2.5 years prior to analysis; the other products appear to have been manufactured in the preceding year. Thus, this finding is consistent with data from the Tewksbury hypochlorite study, providing additional evidence of product "enrichment" with perchlorate over time.

4.3.4. Potential Impacts

Data obtained during this limited investigatory effort suggests that perchlorates are present in hypochlorite solutions used in water and wastewater treatment plants in the range of hundreds to thousands of μg/L, depending upon length and condition of product storage. Similarly, upon purchase in the supermarket, most household bleaches are likely to contain perchlorate in the low to moderate hundreds of μg/Ls - with levels rising into the thousands of μg/L with prolonged storage in the store and/or at a residence.

What are the implications of such a finding?

Drinking Water - There is a large dilution factor in the chlorination processes at water treatment plants. For example, at the Tewksbury plant, 50 gallons of (15%) sodium hypochlorite solution is used to disinfect one million gallons of drinking water, leading to a 20,000 to 1 ratio. Even at the highest perchlorate level of 6750 μg/L, the distributed water would have only 0.34 μg/L perchlorate. However, even this low concentration is now routinely detectable using an LC/MS/MS testing method. Accordingly, absent additional efforts to minimize breakdown of hypochlorite solutions, it would appear that low levels of the perchlorate ion (0.2 to 0.4 μg/L) detected in a drinking water supply disinfected with sodium hypochlorite solutions could be attributable to the chlorination process.

Drinking water impacts may be most pronounced, however, at smaller (non-community) public water supplies. In such cases, solutions of hypochlorite are often purchased in bulk, to keep costs low. Given the relatively low system flowrates and disinfectant usage, this can lead to protracted storage times between product purchase and application, which in turn can lead to increased generation of perchlorate. This phenomenon was recently observed at a small water supply at a school in Boxford, where post-disinfection concentrations of perchlorate exceeded 1 μg/L (ppb).

Of most concern is the potential presence of perchlorate in public water supply systems from the disinfection of raw water that may already have low levels of this contaminant, due to area-wide uses of blasting agents,

fireworks, and other commercial products that contain perchlorates. In such cases, the contribution of perchlorate from the use of the hypochlorite disinfectant is added to an existing "base" level in the raw water, which could result in detectable levels "at the tap" in excess of 1 μg/L (ppb)

Wastewater Plants – Similar to drinking water plants, low levels of perchlorate may be present in treated sewage effluent due to the use of hypochlorite disinfection processes. However, dilution in the receiving water body will in most cases reduce concentrations to less than detectable levels at downstream monitoring or use locations.

Household Bleach – Most household washing machines use between 40 – 45 gallons of water per large load of laundry; newer energy efficient models use between 15 and 20 gallons per large load. Even with the newer models, the dilution of 1 cup of (relatively fresh) bleach into 15 gallons of water will result in a perchlorate concentration of less than 5 μg/L. Dilution in a municipal sewer system would likely reduce these levels well below 1 μg/L. For homes with an on-site sewage disposal system, discharge to and dilution in a conventional (1000 to 2000 gallon) septic tank would likely reduce perchlorate levels to less than 1-2 μg/L. Moreover, beyond dilution effects, limited data obtained by MassDEP suggest nearly complete destruction of perchlorate in an (anaerobic) septic tank (see Section 5.2).

While this would indicate that normal household discharge of bleaches into municipal sewerage or conventional septic systems should not be an environmental issue, there are several scenarios where discharges and/or usage may be of concern, including:

- Homes where washing machine discharge is piped directly to a dry well, and is not diluted/treated via a septic tank/system;
- Laundromats with subsurface wastewater discharges; and
- Homes and businesses that use household bleach to disinfect (private) on-site drinking water wells.

4.4. Perchloric Acid

Perchloric acid has the same unique and desirable properties as perchlorate salts: a powerful oxidizing agent that is at the same time safe to use. While the extent of its use in Massachusetts is not at present known, it is clear that industrial-scale discharges of process wastewaters containing this material has the potential to create significant impacts to groundwater and surface water.

4.4.1. Chemistry of Perchloric Acid

Perchloric Acid is marketed principally as a 72% aqueous solution. At room temperature, this solution is not an oxidizing agent, and can be safely transported and stored. It is only when it is hot and concentrated does it become a powerful oxidizing agent – allowing for chemical engineering reactions and production processes that can be carefully designed and controlled. This property makes it unique among the strong acids. (GFS Chemicals, 2005)

4.4.2. Perchloric Acid Discharger in Northeastern Massachusetts

In August 2004, low levels (1 – 3 µg/L) of the perchlorate ion were first detected in the Town of Tewksbury, MA public water supply system, which draws its water from the Merrimack River, the second largest river in the state. It is noteworthy that this detection coincided with the low-flow conditions of August, in which average daily flow in the Merrimack is 3000 cubic feet per second (CFS), compared to almost 20,000 CFS in April.

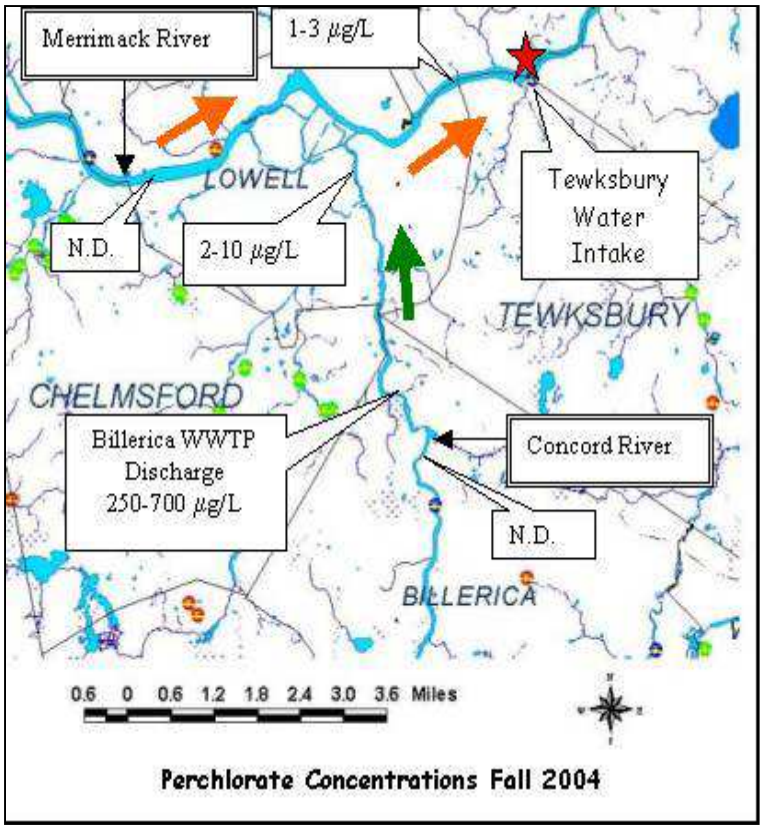


Figure 14: Perchloric Acid Discharge
Concord and Merrimack Rivers, MA

This finding precipitated an effort by MassDEP to locate the source of perchlorate discharge to the river, involving a systematic and iterative sampling program tracking the contaminant upstream of the Tewksbury water intake. Eventually, the source was traced to the discharge from the Town of Billerica Wastewater Treatment Plant, which discharged into the Concord River, a tributary of the Merrimack, over 5 miles upstream of the Tewksbury intake (see Figure 14).

Monitoring of the effluent from the Billerica wastewater plant during September and October 2004 showed consistent levels of perchlorate in the range of 250 to 700 μg/L. The Billerica plant is a secondary treatment system servicing a community of 50,000, with an average daily flow of 3.1 million gallons/day (MGD), including 0.40 MGD of industrial wastewaters. At this average flowrate, approximately 6-10 pounds per day of perchlorates were being discharged from the plant. This was consistent with the 2-4 μg/L concentrations of perchlorate that were being detected in the Concord River downstream of the discharge, where river flowrates varied in the range of 250 to 600 CFS. The highest level detected was 10.3 μg/L of perchlorate on September 7, 2004, when the Concord River flowrate was at its lowest at 142 CFS.

In contrast to the data from the Concord River, mass-flux rates for perchlorate in the Merrimack River "did not add up", leading to speculation that there may have been additional sources of contamination impacting the Tewksbury water intake. Specifically, concentrations of between 1 and 3 μg/L of perchlorate in the Merrimack River at the Tewksbury intake equate to mass flowrates of 20 to 40 pounds/day of perchlorates, given the 2000 to 7000 CFS flowrate in the Merrimack during this time period. Ultimately, this discrepancy was attributed to complex flow patterns in this reach of the Merrimack River that tended to limit the mixing of inflow from the Concord River.

Investigations undertaken by the Town of Billerica eventually identified the (apparent sole) source of perchlorate discharge to the municipal sewerage system: a processor of surgical and medical materials, which was using approximately 220 gallons/month of perchloric acid. Although only a small portion of this acid was discharged (as rinsewater) to the sewer system, it equated to an average of 10 pounds/day of perchlorate. Moreover, perchloric acid use at this facility was via a "batch" operation process, which explained the variability (and spikes) in perchlorate data into and exiting the Billerica wastewater plant. It is noted that this industrial wastewater discharge was not in violation of the facility's permit, as perchloric acid and perchlorate were not (at that time) regulated contaminants in the wastestream.

Currently, this company is treating its wastewater prior to discharge into the Billerica sewerage system, utilizing ion-exchange technology that reduces influent perchlorate concentrations of 2000 mg/L to less than 0.050 mg/L in the company's effluent discharge.

5.0 ANCILLARY FINDINGS

In undertaking the investigations described in this report, MassDEP has made two ancillary findings of relevance to source and occurrence concerns.

5.1. Analytical Testing Procedures

The primary method used to date to test public water supplies for perchlorate in Massachusetts has been EPA Method 314.0, *Determination of Perchlorate in Drinking Water Using Ion Chromatography,* Revision 1.0, November 1999. In using this method, however, MassDEP has specified that laboratories achieve a Reporting Limit of 1 µg/L. This is accomplished by the use of lower concentration spiking solutions and standards, and a series of initial and ongoing quality control requirements and limits. (http://www.mass.gov/dep/brp/dws/files/perchlor.pdf)

MassDEP has conducted 2 rounds of "single blind" Proficiency Test (PT) studies to determine if laboratories are able to comply with method modifications, and achieve a 1 µg/L Reporting Limit. In total, 17 laboratories participated in one or both of these testing efforts, including 7 labs that had demonstrated an initial capability to conduct this procedure ("MassDEP approved labs"). Each study involved a blank sample, and a sample spiked at 1.04 µg/L (first study) and 1.25 µg/L (second study) of perchlorate, at conductivity levels on the high end of Massachusetts' drinking water supplies (approx 500 µS/cm @ 25°C). (http://www.mass.gov/dep/ors/files/perchpt.pdf)

In the first study, 13 of 15 laboratories – including all 7 MassDEP approved labs - successfully analyzed the spiked samples, reporting a perchlorate concentration within +/- 2 standard deviations of the study mean, with a mean recovery of 83% (i.e., biased slightly low). One of the 17 laboratories reported a "false positive" detection of perchlorate in the blank sample, but at a concentration below the 1 µg/L Reporting Limit. The results were similar in the second study, with 13 of 16 laboratories - including all 7 MassDEP approved labs - reporting acceptable results. In the second study, the mean recovery of the (1.25 µg/L) spike was 83.9%, with a standard deviation of 0.116 µg/L.

A subsequent "double blind" study was also conducted by the American Water Works Association of the 7 MassDEP approved laboratories, this time using samples with higher concentrations of dissolved salts (i.e., 1200 µS/cm) more typical of other areas of the country. Despite this challenge, 6 of the 7 MassDEP approved laboratories performed acceptably; the exception being a laboratory located in Arizona that did

little work within Massachusetts, and that reported < 0.3 μg/L perchlorate in all samples not prepared in Reagent Water.

Overall, these data and results enabled the agency to conclude that the use of the MassDEP-modified Method 314.0 is sufficient to achieve a 1 μg/L Reporting Limit on drinking water matrices common in Massachusetts, with a low probability of a false-positive detection above the Reporting Limit.

Field experiences have further supported the validity of this finding. Specifically, in reviewing over 600 analyses of drinking water samples, MassDEP is not aware of a single case of a "false positive" detection above the 1 μg/L Reporting Limit, provided all specified steps and methodological modifications are followed.[1] Split samples conducted on approximately 30 drinking water samples have demonstrated good correlation between the MassDEP-modified EPA Method 314.0 and an LC/MS/MS procedure (draft EPA Method 331.0). In a few cases, matrix interference in a drinking water sample (e.g., raw water sample from the Merrimack River) precluded quantitation by EPA 314.0; however, QC requirements in the modified method (i.e., retesting/spiking samples with detects above 0.8 μg/L) clearly revealed the condition of concern, leading to further retesting by LC/MS/MS.

Although MassDEP-modified EPA Method 314.0 has performed well for its intended application in Massachusetts (i.e., analysis of drinking water with relatively low dissolved salts), it cannot provide definitive identification and quantification of the perchlorate ion, and cannot be relied upon to quantitate levels of perchlorate less than 1 μg/L. It is for this reason that MassDEP has used an LC/MS/MS technique to verify positive results from a Method 314.0 analysis, as well as conduct testing/verification testing of wastewater, hypochlorite, and other non-drinking water matrices.

5.2. Perchlorate Treatment in Septic Tanks

In investigating sources and impacts of perchlorate contamination, MassDEP began to consider the degree of treatment that might occur in conventional septic systems. This interest was catalyzed by two specific issues and concerns:

- The fact that low-levels of perchlorate were likely being discharged into numerous residential septic systems (via use and discharge of household bleach) which could lead to pervasive low-level groundwater contamination in areas without central sewerage systems; and

[1] A suspected false positive report for an un-named reservoir in Springfield was later found by MassDEP to be a laboratory error

- The likely treatment of perchlorate-contaminated residential (private) drinking water wells by a Reverse Osmosis system, which would lead to a concentrated wastestream discharge to on-site septic systems (i.e., would this just be transferring the problem back to the groundwater?)

A number of researchers (e.g., Urbansky) have published materials on the anaerobic degradation/treatment of perchlorates. With this in mind, MassDEP had the opportunity to obtain septic tank effluent samples at two locations where the potable water source was contaminated with high concentrations of the perchlorate ion. Details and data in this regard are provided in Table 8.

Table 8: Treatment of Perchlorate in a Septic Tank

Town	Description	Date	Perchlorate Concentration by LC/MS/MS (µg/L)	
			Tap Water	Septic Tank Effluent
Boxboro	Condominiums	10/19/04	Approx 850*	0.23
Westford	Private Home	12/02/04	190	N.D. @ 0.2 µg/L RL

* 783 µg/L on 10/7; 943 µg/L on 10/22

As can be seen, the influent perchlorate ion is being almost completely degraded by the highly reducing conditions present within the septic tank environments. What is particularly noteworthy is the situation in Boxboro, where the septic tank in question was in the process of being decommissioned because of overload. Specifically, this 5000-gallon tank was receiving on average 3000 gallons/day of sewage from a block of buildings within a condominium complex – resulting in less than 48 hours of residence time.

6.0 CONCLUSIONS

On the basis of information and data obtained during the last 12 months, MassDEP has reached the following conclusions and tentative findings:

Occurrence – The perchlorate ion is not pervasive in surface waters or groundwater in Massachusetts, at a Reporting Limit of 1 µg/L (ppb). However, localized impacts exist at certain sites, creating conditions that can pose significant health risks to impacted populations.

Sources – Military products and operations have caused significant and extensive groundwater impacts in Massachusetts, creating long plumes containing hundreds of μg/L (ppb) of perchlorate. The most significant non-military sources of perchlorate contamination encountered to date in Massachusetts have been an industrial user of perchloric acid, and blasting operations that had used (or likely used) perchlorate-containing explosive materials. Lesser (though still locally problematic) sources have included fireworks displays and hypochlorite/bleach solutions.

Blasting Operations – Certain Emulsion and Water Gel Blasting Agents contain perchlorate salts, typically in the range of 5% – 15% by weight, but sometimes higher. It is theorized that misfires and/or "bad housekeeping" associated with the use of these products are the primary mechanisms that result in groundwater impacts, which can be in the hundreds or even thousands of μg/L (ppb) of perchlorate.

Fireworks – It would appear that potassium perchlorate salts have been increasingly used in pyrotechnic products in the last 10-15 years, because of their superior ability to produce vivid colors in aerial display shells. Atmospheric fallout of combustion particulates and, perhaps more importantly, un-combusted debris, result in localized groundwater impacts. These impacts range from tens of μg/L (ppb) of perchlorate locally for larger and more recent displays, to single digit concentrations in downgradient areas and/or for smaller or more historical launchings.

Hypochlorite/Bleach Solutions – Hundreds to thousands of μg/L (ppb) of perchlorate has been documented in commercial and household hypochlorite (bleach) solutions, with perchlorate concentrations increasing as a function of storage time, temperature, and ionic strength. It is theorized that perchlorate formation in these solutions is related to the formation of chlorates, a well-known hypochlorite decomposition by-product. The use of perchlorate-containing hypochlorite solutions at water treatment plants could lead to concentrations of perchlorate in the water supply distribution systems in the range of 0.2 to 0.4 μg/L.

7.0 RECOMMENDATIONS

It is recommended that regulators and industry further study and better understand the conditions and mechanisms that lead to the perchlorate releases and/or impacts discussed in this report, with the overall goal of preventing, minimizing, and/or mitigating impacts to human health and the environment.

Blasting Operations

1. Manufacturers of explosive materials should clearly indicate the percentage of perchlorate salts in their products.
2. Contractors and regulators should be mindful of the environmental sensitivity of blasting sites when using perchlorate-containing explosive materials, particularly if drinking water supply wells are located nearby. Additional guidance in this regard is available at http://www.mass.gov/dep/bwsc/files/blasting.htm.
3. Blasting contractors should make every reasonable effort to prevent misfires from occurring when using perchlorate-containing materials, and, in the event of a misfire, should ensure that all reasonable steps are taken to recover un-detonated materials.

Fireworks

1. Manufacturers and/or distributors should clearly indicate the percentage of perchlorate salts in their products.
2. Contractors, regulators, and display organizers should be mindful of the environmental sensitivity of launch areas, particularly if drinking water supply wells are nearby. All areas at and downwind of the launch area should be thoroughly surveyed following a display (and/or at first light) to identify and remove debris and fallout.

Hypochlorite/Bleach Solutions

Industry should further test and characterize hypochlorite solutions and, based on the results, consider taking necessary and practical steps to prevent the formation of perchlorates in stored materials. Based upon our limited data, improved or enhanced filtering of hypochlorite products may be beneficial to remove the impurities that may be catalyzing the production of chlorates and perchlorates.

8.0 RESEARCH NEEDS

Additional research is needed to further characterize sources, occurrences, and exposures to perchlorate. On the basis of the findings of this document, and other research efforts in this area, the following investigatory projects are suggested:

- *Swimming pools* – Investigate concentrations of perchlorate in swimming pools treated with hypochlorite products.

- *Private Drinking Water Wells* – Determine perchlorate residuals in wells that have been "shocked" and/or are systematically disinfected by hypochlorite products, with a goal toward developing Best Management Practices to minimize concerns in this regard.

- *Fireworks* – Investigate impacts of fireworks displays on ambient air, with respect to particulate fallout to soil, groundwater, and surface waters, as well as inhalation exposures to the viewing and general public.

- *Municipal Landfills* – Test leachate to determine perchlorate content, given the increasing use of perchlorate salts in common household and commercial products.

- *Roadway Flares* –Test monitoring wells and/or surface water runoff near major highways, to ascertain contribution of perchlorate to the environment from use (and discarding) of roadway flares.

List of References

AMEC Earth and Environmental, Inc., *Draft Northwest Corner Data Summary Report*, Impact Area Groundwater Study Program (Massachusetts Military Reservation), January 22, 2004. (http://www.mmr.org/)

Chemical and Engineering News, *What's that stuff: Fireworks*, Volume 79, Number 27, page 30, July 2, 2001 (http://pubs.acs.org/cen/whatstuff/stuff/7927sci3.html)

GFS Chemicals, *Perchlorate Compounds and Perchloric Acid Information by GFS Chemicals*, 2005 (http://www.gfschemicals.com/productcatalog/perchlorate_information.asp

Gordon, Gilbert, and Bubnis, Bernard, *Bleach Stability and Filtration*, AWWA Water Quality Technology Conference, November, 1996, Boston, MA (http://www.powellfab.com/products/SodiumHypo/sodium_hypochlorite_stability_and_filtration.html)

Institute of Manufacturers of Explosives (IME), correspondence from Susan Flanagan to Paul Locke, MassDEP, December 1, 2004

Institute of Manufacturers of Explosives (IME), correspondence from Susan Flanagan to John Fitzgerald, MassDEP, April 20, 2005

Powell Fabrication & Manufacturing Inc., *Sodium Hypochlorite General Information Handbook*, Updated April 8, 2002 (http://www.powellfab.com/products/products_sodium_info.html)

Schumacher, JC, *Perchlorates: Their Properties, Manufacture and Uses*, American Chemical Society Monograph Series, Reinhold Publishing Corporation, NY (1960)

Urbansky, Edward Todd, *Perchlorate as an Environmental Contaminant*, Environmental Sci & Pollution Res, **9** (3) 187-192 (2002) (http://clu-in.org/download/contaminantfocus/perchlorate/ESPR_9_187_192.pdf)

Zediana, Lewis and Ladderbush, Marlene, Tewksbury Department of Public Works, Memorandum to File, 11/10/04

History of Revisions

Date	Section	Change
April 2006	4.2	Added new Section 4.2.5, "Easthampton Fireworks Display". New data is provided for a "community event" fireworks launch site. These data are consistent with modeled expectations and empirical data from other similar sites. Moreover, upstream samples in a receiving waterway suggest watershed "background" value of perchlorate of approximately 0.2 μg/L.
	4.3.4	New information and data provided on a small water supply system servicing a school in Boxford. Of significance is the observation that small water supplies may be most at risk for perchlorate impacts, given (a) low-level concentrations of perchlorate in (localized) sources waters, (b) the prevalent use of hypochlorite solutions as a disinfectant, (c) the low-usage rate of the hypochlorite solution (that can lead to long storage times with a concomitant build-up of perchlorate in the hypochlorite solution), and (d) infrequent cleanouts of the hypochlorite tanks.

EXHIBIT C

Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 567 (2006) 33–38
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Potential perchlorate exposure from *Citrus* sp. irrigated with contaminated water

C.A. Sanchez [a,*], R.I. Krieger [b], N.R. Khandaker [a], L. Valentin-Blasini [c], B.C. Blount [c]

[a] *Department of Soil, Water, and Environmental Sciences, Yuma Agricultural Center, The University of Arizona, Yuma, AZ 85364, USA*
[b] *Personal Chemical Exposure Program, Department of Entomology, University of California, Riverside, Riverside, CA 92521, USA*
[c] *National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), Atlanta, GA 30341, USA*

Received 16 November 2005; received in revised form 3 February 2006; accepted 6 February 2006
Available online 6 March 2006

Abstract

Citrus produced in the southwestern United States is often irrigated with perchlorate-contaminated water. This irrigation water includes Colorado River water which is contaminated with perchlorate from a manufacturing plant previously located near the Las Vegas Wash, and ground water from wells in Riverside and San Bernardino counties of California which are affected by a perchlorate plume associated with an aerospace facility once located near Redlands, California. Studies were conducted to evaluate the uptake and distribution of perchlorate in citrus irrigated with contaminated water, and estimate potential human exposure to perchlorate from the various citrus types including lemon (*Citrus limon*), grapefruit (*Citrus paradise*), and orange (*Citrus sinensis*) produced in the region. Perchlorate concentrations ranged from less than 2–9 μg/L for Colorado River water and from below detection to approximately 18 μg/L for water samples from wells used to irrigate citrus. Destructive sampling of lemon trees produced with Colorado River water show perchlorate concentrations larger in the leaves (1835 μg/kg dry weight (dw)) followed by the fruit (128 μg/kg dw). Mean perchlorate concentrations in roots, trunk, and branches were all less than 30 μg/kg dw. Fruit pulp analyzed in the survey show perchlorate concentrations ranged from below detection limit to 38 μg/kg fresh weight (fw), and were related to the perchlorate concentration of irrigation water. Mean hypothetical exposures (μg/person/day) of children and adults from lemons (0.005 and 0.009), grapefruit (0.03 and 0.24), and oranges (0.51 and 1.20) were estimated. These data show that potential perchlorate exposures from citrus in the southwestern United States are negligible relative to the reference dose recommended by the National Academy of Sciences.

Keywords: Lemon (*Citrus limon*); Grapefruit (*Citrus paradise*); Orange (*Citrus sinensis*); Colorado River; Perchlorate

1. Introduction

Perchlorate has been discovered in surface and ground water supplies throughout the United States. There is concern that these perchlorate-contaminated waters may represent a health risk both as sources of drinking water and irrigation water for food crops. Perchlorate has the potential to cause thyroid dysfunction by inhibiting iodide uptake by the sodium iodide symporter (NIS) [1].

Perchlorate has been detected in several non-crop plant species in non-cultivated ecosystems exposed to aerospace and defense-related perchlorate contamination [2–5]. Accumulation of perchlorate in tobacco [6] fertilized with perchlorate-containing Chilean nitrate [7,8] is also documented. A number of studies have shown perchlorate accumulation in edible leafy vegetables irrigated with perchlorate-contaminated water [9–11]. Data also indicate potential perchlorate accumulation in fruiting and seed crops irrigated with contaminated water but bioconcentration appears lower compared to leafy vegetation [12].

A substantial area of citrus is irrigated with perchlorate-contaminated water in the southwestern United States. Citrus produced in the lower Colorado River valleys of Arizona and California and the Coachella Valley of California are irrigated with Colorado River water, which has had perchlorate concentrations ranging from 5 to 9 μg/L [13]. Approximately 5 billion m^3 of water are diverted at the Imperial Diversion Dam to irrigated crops in southwestern Arizona and southern California. Perchlorate contamination in the Colorado River is introduced into Lake Mead by a perchlorate salt manufacturing plant previously located near the Las Vegas Wash.

* Corresponding author. Tel.: +1 928 782 3836; fax: +1 928 782 1940.
E-mail address: sanchez@ag.arizona.edu (C.A. Sanchez).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.02.013

Citrus produced in portions of Riverside and San Bernardino counties of California outside the low desert are irrigated with wells affected by a perchlorate ground water plume associated with an aerospace facility near Redlands, California. The objectives of this study were to evaluate the uptake and distribution of perchlorate in *Citrus* sp. irrigated with contaminated water, and estimate potential human exposure to perchlorate from the various citrus types produced in the region.

2. Experimental

2.1. Uptake and distribution

These samples were actually generated from another study aimed at evaluating the redistribution of ^{15}N-labeled nitrogen in young citrus. Nine five-year-old lemon "Limoneira 8A Lisbon" on "Volkamariana" rootstock at the Yuma Mesa Agricultural Center were sacrificed for these evaluations. These trees were destructively sampled December 5, 2001. All leaves and fruit were hand harvested from each tree. The branches were then removed with a saw from the trunk of the tree. The whole fruit (peel and pulp) was cut into wedges and the branches were cut further into small segments. All leaves, fruit wedges, and branch segments were labeled appropriately, and placed in an oven for drying. The stumps and roots of each tree were pulled out of the ground with a tractor and chain, labeled, and transported to an open storage area for air-drying.

The leaves and fruit wedges were ground directly after drying. The branch segments were ground after processing through a wood chipper. Following 4 months of air-drying, the trunks and roots were separated and processed for grinding. Because trunk segments caused the mechanical failure of two wood chippers in rapid succession, we improvised another approach for processing the trunk and root. Trunk and roots were cut at short intervals (approximately 5 cm) with a chain saw and wood shavings were collected and composited for each tree, and dried in an oven. This composite sample was ground for analysis.

3. Survey of fruit and leaves

Citrus samples were collected during harvest season from fields across southwestern Arizona and southern California during 2004–2005. Samples were collected from different types of citrus including lemon (*Citrus limon*), grapefruit (*Citrus paradise*), and orange (*Citrus sinensis*). The number and location of samples were reflective of the commercial industry. The majority of citrus produced in the lower Colorado River valleys are lemons, with modest orange production, and no commercial grapefruit products. All lemon samples, and a few orange samples, were collected in this area. The only grapefruit collected in this area was from the University of Arizona Research Farm near Yuma, Arizona. Most of the citrus produced in the Coachella Valley, and in the higher altitude regions of southern California, are oranges with modest grapefruit production. It was from this area we collected most orange and grapefruit samples. Lemon, orange, and grapefruit samples were also collected from an orchard in Los Angeles County, suspected of being irrigated with water affected by a perchlorate plume. For each sample we attempted to collect 10 fruits at random from each orchard. For a subset of these we collected corresponding leaf samples from the trees. For all fruit samples, peel and pulp were separated by hand and the leaves, peel, and pulp were frozen separately. The frozen samples were freeze-dried on a Labconco freeze drier. Freeze-drying of leaf and peel tissue typically was complete within 48 h but pulp tissue often required 96 h. Weights before and after freeze-drying were recorded and the samples were subsequently ground and stored in vials for extraction.

3.1. Extraction of perchlorate from plant material

We used an extraction procedure described previously [14] with minor modifications. Briefly, 600 mg of freeze-dried product was weighed into centrifuge tubes and 15 mL of DI water were added. The tubes were boiled for 30 min and the contents were placed in a refrigerator overnight with occasional gentle shaking. The tubes were then centrifuged for 30 min and the supernatants filtered sequentially through Kim wipes and 0.2 μm Gelman ion membrane syringe filters. Two milliliter of the above extract (extract 1) was reacted with 1000 mg DD6 alumina. Vials were gently agitated two or three times over a 24-h period after which 18 mL of DI water was added to the mixture. After stirring and settling, this solution was filtered through another 0.2 μm Gelman ion membrane syringe filter and the resulting solution was labeled "extract 2". This sample was stored in the freezer until analysis by ion chromatography with conductivity detection (IC-CD). Before loading on the IC-CD, the extracts were allowed to reach room temperature and were filtered through pre-conditioned Dionex "On Guard" RP syringe filters. Furthermore, the first 0.75 mL of sample (extract 2) pushed through the filter was discarded and the remaining aliquots used for IC-CD analysis.

3.2. Perchlorate analysis

Perchlorate analyses were initially performed by IC-CD using a Dionex 2500 described previously [11]. Briefly, this unit consists of an IP 25 isocratic pump, an EG50 eluent generator, a continuous regenerating trap column, a CD 25 conductivity detector, the 2 mm AG16/AS16 guard and separation column pair, and an AMMS III suppressor. The columns, suppressor, and detector are housed in an LC 30 chromatography oven. We used 50 mM KOH eluent and 50 mM sulfuric acid suppression. A minimum of 10% of the samples were extracted with a 100 μg/L perchlorate standard to yield 10 μg/L perchlorate standard addition after dilution. The method detection limit (MDL) was determined using the procedure outlined in EPA method 314.0 [15] using seven replicates of a standard in reagent water. The calculated MDL was 0.2 μg/L using a 0.5 μg/L standard. We set the minimum reporting level (MRL) for citrus plant extracts at 1.5 μg/L. As a standard practice we ran 10% duplicate extractions in addition to the 10% spiked additions. Duplicate aliquots

of a given extraction were always analyzed. We generally repeated analysis if recovery of standards and standard additions was less than 85% and variation among duplicates exceeded 25%.

Branch, trunk, and fruit tissue were below detection by IC-CD and root tissue gave false positive perchlorate peaks by IC-CD. Accurate quantification of these tissues required IC/MS/MS. Perchlorate concentrations measured in leaves by IC-CD and IC/MS/MS agreed closely but a few leaf extracts produced co-eluting peaks making accurate integration difficult. Leaf sample extracts with problematic matrices, those with co-eluting peaks, and several samples at random were sent out for IC/MS/MS analysis. Therefore, all root, trunk, branch and fruit tissues from the destructive sampling study, all fruit pulp from the survey, a selected subset of peel samples from the survey, and approximately 25% of all leaf samples collected, were sent to a laboratory for analysis by IC/MS/MS using an ^{18}O internal standard methodology similar to that reported by others [16]. Briefly, 0.5 mL of aqueous sample extract was spiked with an isotopically labeled internal standard ($Cl^{18}O_4^-$) and diluted 1:1 with deionized water. This solution was subsequently analyzed using ion chromatography–electrospray ionization–tandem mass spectrometry. Perchlorate was quantified based on the peak area ratio of analyte to stable isotope-labeled internal standard. A subset of samples (10%) were analyzed further using standard addition, and produced acceptable percent differences of <10%. Absolute assay accuracy was verified by the blind analysis of four different perchlorate reference solutions (AccuStandard, New Haven, CT, USA); analysis of these proficiency testing solutions across the study time period yielded an average percent difference of −5.2% (CI −7.2 to −3.2%). The MDL was estimated to be 0.02 μg/L and the MRL was 0.1 μg/L.

The MRL would be approximately 375 μg/kg dw by IC-CD and 25 μg/kg dw by IC/MS/MS using our extraction ratio. Dry matter content ranged from 33 to 98% for leaves, 14 to 30% for peels, and 8 to 17% for fruit pulp. Therefore, the MRL levels by IC-CD would be approximately 190, 75, and 38 μg/kg fw, for leaves, peel, and pulp, respectively. Reporting levels by IC/MS/MS would be approximately 13, 5, and 2.5 μg/kg fw for leaves, peel, and pulp, respectively.

3.3. Perchlorate concentration in irrigation water

Aliquots of composite Colorado River water samples, collected by the U.S. Bureau of Reclamation (USBOR) at the Imperial Diversion Dam, from March 2003 through September 2005, were analyzed for perchlorate in our laboratory. Water samples from wells and reservoirs used for irrigation were also collected at the time of citrus sampling. These water samples were analyzed for perchlorate using EPA Method 314.0 [15]. We estimated a reporting level of 1 μg/L in water using methods described above. Perchlorate concentrations of Colorado River at the Imperial Dam were compared to samples collected up-stream at Willow Beach by the Nevada Division of Environmental Protection from December 1999 through April 2005 [17].

3.4. Exposure estimates

An MRL of 0.1 μg/L by IC/MS/MS would correspond to approximately 2.5 μg/kg fw for fruit pulp. For values below MRL, we used estimates of 1.25 μg/kg fw and for values below detection we used estimates of 0.625 μg/kg fw. We used median perchlorate concentrations in the edible fruit pulp and mean and 95th percentile consumption estimates [18] to estimate exposures.

4. Results and discussion

Perchlorate concentrations of the Colorado River ranged from 1 to 9 μg/L (Fig. 1). Data were collected by the Nevada Department of Environmental Protection at Willow Beach, 11 miles down stream of Lake Mead, are shown from late 1999 through April 2005. We did not begin collecting data at Imperial Diversion Dam, 290 miles downstream of Lake Mead, until March 2003. There was some temporal variation in perchlorate concentrations between the two sampling locations which is not surprising considering that water travel times, water quantity, and water quality are all potentially altered by diversion dams, storage reservoirs, and tributaries along the river. Nevertheless, the data generally compare favorably where the average concentrations from March 2003 through April 2005 were 4.1 and 4.0 μg/L at Willow Beach and Imperial Diversion, respectively. Thus, where we do not have data for the Imperial Diversion Dam, we used data from Willow Beach as a reasonable estimate of perchlorate concentrations of irrigation water. Studies have shown that perchlorate is not physically or chemically retained by soil [19,20]. Thus, perchlorate is largely transported into and through soils with irrigation water and the perchlorate concentration of this water is the most reliable estimate of plant available perchlorate over a growing season.

The concentrations of perchlorate in other water sources used to irrigate citrus ranged from below detection from well water in Los Angeles County and some reservoirs and wells in the Coachella Valley to18 μg/L from a well in Loma Linda, near

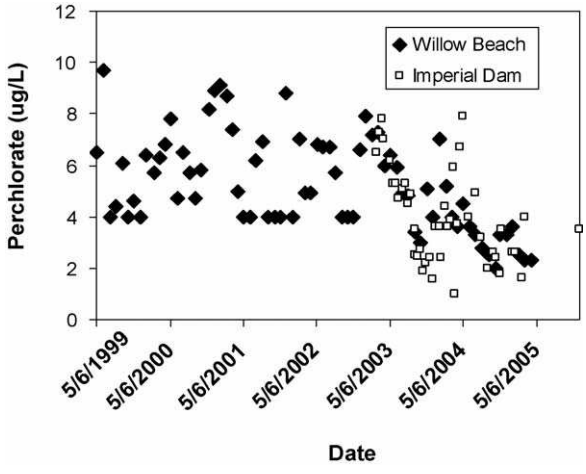


Fig. 1. Perchlorate concentration in Colorado River over study period.

Redlands (Table 1). It should be noted that some citrus in the Coachella Valley is irrigated with surface deliveries from the Colorado River, some citrus is irrigated with ground water, and some is irrigated with both sources. It has been alleged that ground water in the Coachella Valley has been contaminated with perchlorate from recharge from the Colorado River [21] and it is debated whether this is from an intentional recharge program administered by the irrigation district or incidental recharge through agricultural irrigation. Colorado River water transported through the aqueduct has also been used to recharge ground water along its route from the Colorado River, near Parker, to Los Angeles and the river might have contributed toward the perchlorate contamination of other ground water sources used to irrigate citrus. Trace levels of perchlorate were found in the fruit from some orchards in the Coachella Valley where the corresponding water samples tested below detection by IC-CD. It is likely these orchards are irrigated with other sources of water in addition to the water collected at the time of sampling. Furthermore, previous studies have shown perchlorate in rainfall [22] and bottled water [23] at sub part per billion levels and we cannot rule out the presence of perchlorate below our detection by IC-CD. However, for the orchard in Los Angeles County we found no detectable perchlorate in lemon, orange, and grapefruit, where the only source of water was a well where perchlorate was below detection by IC-CD.

We do not consider fertilizer a likely source of perchlorate in the citrus samples collected. As noted previously, the only fertilizer source with a significant perchlorate content is Chilean nitrate [8]. More than one of the authors work closely with citrus producers in the western United States and could identify no situations where Chilean nitrate was used in recent history. A review of the scientific literature show some use of Chilean nitrate in N fertilizer experiments initiated in the 1920s [24,25] but could identify no use in several other fertilizer N experiments conducted from the 1950s through more recent times [26–28]. Some low biuret urea is used for foliar fertilizer of citrus trees [29]. This history suggest that Chilean nitrate was used by some producers decades ago but its use was discontinued as other more economical N fertilizer sources became available through the Haber process. As a result of large leaching fractions of irrigation waters used in the western United States non-reactive anion, such as perchlorate would be expected to leach out of the crop-rooting zone within a season after application [19,20].

Table 2
Perchlorate concentrations of various tree parts for destructively sampled lemon trees

Tree part	Perchlorate (μg/kg dw)[a]	
	Range	Mean
Roots	<DL–55	<MRL
Trunk	<DL–<MRL	<MRL
Branches	<DL–65	26
Leaves	699–4931	1835
Fruit	64–195	128

[a] MRL is minimum reporting level and DL is detection limit.

The average perchlorate concentrations (μg/kg dw) in lemon trees irrigated with Colorado River water are shown in Table 2. Perchlorate in the trunk was below MRL and perchlorate in the roots and branches was close to MRL by IC/MS/MS. Perchlorate concentrations in the fruit (peel and pulp) and leaves were 128 and 1835 g/kg dw, respectively. The trees were 5-years-old and it is estimated they were irrigated with water having an average perchlorate close to 6 μg/L. Water consumption of an individual citrus tree can range from 80 to 100 m^3 annually [30] and citrus retains leaves for 2–3 years [31]. Thus, there is a large potential for perchlorate accumulation in these transpiring leaves through xylem transport where citrus is irrigated with contaminated water.

These data are generally consistent with data collected in the survey, which show much larger accumulations in the leaves compared to the fruit (Tables 3 and 4). The larger variation in concentration in leaves collected in the survey is likely the result of varying perchlorate concentrations of water sources and varying age of leaves sampled. The trees that were destructively sampled were all of the same age, adjacent in the same field, irrigated with the same Colorado River water over the same time interval, and our sample represented a composite of all the leaves on the tree. For the survey we sampled trees of varying age

Table 1
Perchlorate concentration of various water sources used to irrigate citrus

Location	County/state	Date collected	Perchlorate (μg/L)[a]
Coachella Valley	Riverside Co., CA, USA	June 30, 2004	4.1
Loma Linda	San Bernardino Co., CA, USA	December 7, 2004	18.1
Riverside	Riverside Co., CA, USA	January 4, 2005	3.4
Riverside	Riverside Co., CA, USA	February 14, 2005	1.0
Riverside	Riverside Co., CA, USA	February 14, 2005	2.1
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	<DL
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	2.7
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	<DL
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	<DL
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	11.4
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	11.6
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	2.5
Loma Linda	San Bernardino Co., CA, USA	August 20, 2005	15.8
Canoga Park	Los Angeles Co., CA, USA	October 13, 2005	<DL

[a] DL is detection limit.

Table 3
Concentrations of perchlorate in leaves and peel samples collected in survey

Crop	*n*	Dry weight (μg/kg)			Fresh weight (μg/kg)		
		Minimum	Maximum	Mean	Minimum	Maximum	Mean
Leaves							
Lemon	11	567	4979	2357	283	3629	1695
Grapefruit	4	372	4346	1659	145	1738	647
Orange	8	894	8987	2875	430	4494	1424
Peel							
Lemon	5	29	261	115	5	41	18
Grapefruit	4	17	149	80	4	29	17
Orange	12	89	731	199	22	189	48

Table 4
Hypothetical mean and 95th percentile perchlorate exposure of children and adults who consume citrus

Crop	*n*	Perchlorate (μg/kg fw)			Citrus consumption (g/day)		Exposure (μg/day)[b]	
		Range	Mean[a]	Median	Children[a]	Adult[a]	Children[a]	Adult[a]
Lemon	33	<DL–14.8	2.3 (6.1)	1.3	4 (27)	7 (50)	0.005 (0.035)	0.009 (0.065)
Grapefruit	15	<DL–16.2	3.3 (8.1)	1.3	24 (121)	185 (703)	0.03 (0.16)	0.24 (0.91)
Orange	28	<DL–37.6	7.4 (25.3)	4.8	107 (323)	249 (744)	0.51 (1.55)	1.20 (3.57)

[a] Values in parenthesis represent 95th percentile numbers.
[b] Exposure estimates calculated by (median perchlorate content, μg/kg fw) × (mean (or 95th percentile) consumption estimates, kg).

(7–30-years-old), leaves were collected at random from the tree canopy, and we did not distinguish leaf age. The larger values for perchlorate concentration in all tissues are generally associated with the trees sampled at Loma Linda.

Perchlorate concentrations were notably lower in the fruit peel and pulp compared to the leaves (Tables 3 and 4). Concentrations in the fruit pulp ranged from below detection in an orchard in Los Angeles County to 38 μg/kg fw at Loma Linda. Because the initial sample from Loma Linda appeared to be an outlier compared to other samples, we collected additional samples 6 months later, and obtained similar results (water 16 μg/L and fruit pulp 29 μg/kg). Water transpiration through fruit tissue is less than the leaves and a significant portion of the accumulated solutes in the fruit are transported through phloem transport [32]. Although we are inclined to assume much less perchlorate is translocated to the fruit, compared to the leaves, we cannot rule out biochemical reduction of the perchlorate which has been identified as being important in certain plant species [33,34].

Mean hypothetical adult perchlorate exposure in the edible fruit averaged 0.009, 0.23, and 1.20 μg/day for lemons, grapefruit, and oranges, respectively (Table 4). Similar results for children averaged 0.005, 0.03 and 0.51 μg/day. It should be noted that these estimates for oranges include those samples collected at Loma Linda, which is a private orchard and this citrus is not marketed commercially. Estimated dosages for a 70 kg adult [35] from oranges would be 0.02 μg/kg bw which is less than 5% of the no effect reference dose of 0.7 μg/kg recommended by the National Academy of Sciences (NAS). Estimating dosage for children are more difficult because consumption data are limited and our consumption estimate includes a wide range of children's ages and body weights. However, even considering a child with a 10 kg body weight, the estimated dosage would be approximately 10% the NAS-recommended reference dose. The NAS reference dosage is based upon a no-observed effect level of 7 μg/kg from human iodide uptake studies [36] to which a 10-fold uncertainty factor was applied to address all potentially sensitive subpopulations [37].

It is important to note that from previous work with leafy vegetables [11,38] we obtained reasonable estimates of exposure by IC-CD using estimated values below levels of quantification and detection. If we had used a similar approach for citrus and relied on IC-CD analysis only, we would have overestimated perchlorate exposure by a factor of 4. For crops like citrus, where perchlorate accumulation is low but human consumption is high, accurate estimates of exposure require sensitive and selective analytical methodology such as IC–MS/MS.

In conclusion, citrus trees do accumulate perchlorate from low concentrations in irrigation water. There is a potential for high perchlorate concentrations to accumulate in transpiring leaves but only trace levels are found in the edible fruit. These data show that potential perchlorate exposures from citrus in the southwestern United States are small relative to the reference dose recommended by the NAS.

Acknowledgements

Funding for this study was provided by grants from the California Citrus Research Board and the USDA-National Integrated Food Safety Program. We are also grateful to the countless citrus producers in the region, who allowed us access to their production fields.

References

[1] J.J. Clark, in: E.T. Urbansky (Ed.), Perchlorate in the Environment, Kluwer/Plenum, New York, NY, 2000.
[2] E.T. Urbansky, M.L. Magnuson, C.A. Kelty, S.K. Brown, Sci. Total Environ. 256 (2000) 227.
[3] P.N. Smith, C.W. Theodorakis, T.A. Anderson, R.J. Kendall, Ecotoxicology 10 (2000) 305.
[4] K. Tan, T.A. Anderson, M.W. Jones, P.N. Smith, W.A. Jackson, J. Environ. Qual. 33 (2004) 1638.
[5] P.N. Smith, L. Yu, T. McMurry, T.A. Anderson, Environ. Pollut. 132 (2004) 121.
[6] J.J. Ellington, N.L. Wolfe, A.W. Garrison, J.J. Evans, J.K. Avants, Q. Teng, Environ. Sci. Technol. 35 (2001) 3213.
[7] E.T. Urbansky, S.K. Brown, M.L. Magnuson, C.A. Kelty, Environ. Pollut. 112 (2001) 299.
[8] E.T. Urbansky, T.W. Collette, W.P. Robarge, W.L. Hall, J.M. Skillen, P.F. Kane. Final Report. (2001) EPA/600/R-01/047.
[9] EWG (2003). http//www.efg.og/reports/rocketlettuce/.
[10] FDA (2004). www.cfsan.fda.gov/∼dms/clo4data.html.
[11] C.A. Sanchez, R.I. Krieger, R.N. Khandaker, R.C. Moore, K.C. Holts, L.L. Neidel, J. Agric. Food Chem. 53 (2005) 5479.
[12] W.A. Jackson, P. Joseph, P. Laxman, K. Tan, P.N. Smith, L. Yu, T.A. Anderson, J. Agric. Food Chem. 53 (2005) 369.
[13] DHS. Department of Health Services, Sacramento, California (2000) www.dhs.cahwnet.gov/org/ps/.
[14] J.J. Ellington, J.J. Evans, J. Chromatagr. 898 (2000) 193.
[15] U.S. EPA. U.S. EPA Method 314.0. U.S. Environmental Protection Agency. National Exposure Research Laboratory and Office of Research and Development, Cincinnati, OH 45268.
[16] L. Valentin-Blasini, J.P. Mauldlin, D. Maple, B.C. Blount, Anal. Chem. 77 (2005) 2475.
[17] Nevada Division of Environmental Protection. http://ndep.nv.gov/bca/file/perchlorate_willow_beach.xls.
[18] H. Smiciklas-Wright, D.C. Mitchell, S.J. Mickle, A.J. Cook, J.D. Goldman, J. USDA NFS Report No. 96-5, 2002.
[19] E.T. Ubransky, S.K. Brown, J. Environ. Monit. 5 (2003) 1.
[20] D.K. Tipton, D.E. Rolston, K.M. Scow, J. Environ. Qual. 32 (2003) 40.
[21] C. Hogue, Chem. Eng. News 81 (33) (2003) 37.
[22] P.K. Dasgupta, P.K. Martinelango, W.A. Jackson, T.A. Anderson, K. Tian, R.W. Tock, S. Rajagopalan, Environ. Sci. Technol. 39 (2005) 1569.
[23] S.A. Snyder, B.J. Vanderford, D.J. Rexing, Environ. Sci. Technol. 39 (2005) 4586.
[24] G.A. Cahoon, E.S. Morton, W.W. Jones, M.J. Garber, Proc. Am. Soc. Hort. Sci. 74 (1959) 289.
[25] W.W. Jones, C.B. Cree, T.W. Embleton, Proc. Am. Soc. Hort. Sci. 77 (1961) 146.
[26] D.R. Rodeny, G.C. Sharples, Proc. Am. Soc. Hort. Sci. 78 (1962) 185.
[27] R.H. Hilgeman, C.W. Van Horn, University of Arizona Agricultural Experimental Station Bull, p. 258.
[28] W.W. Jones, T.W. Embleton, S.B. Boswell, J. Am. Soc. Hort. Sci. 95 (1970) 46.
[29] C.J. Lovatt, Hort. Technol. 9 (1999) 9.
[30] L.J. Erie, O.F. French, D.A. Bucks, K. Harris, Conservation Research Report Number 29. (1982), United States Department of Agriculture, Agricultural Research Service, Washington, DC.
[31] P. Spiegel-Roy, E.E. Goldschmidt, Cambridge University Press, Cambridge, UK, 1996, p. 230.
[32] A. Garcia-Luis, M.E.M. Oliverira, Y. Bordon, D.L. Siqueira, S. Tominaga, J.L. Guardiola, Ann. Bot. (2002) 755.
[33] V.A. Nzengung, C. Wang, G. Harvey, Environ. Sci. Technol. 33 (1999) 1470.
[34] B. Van Aken, J.L. Schnoor, Environ. Sci. Technol. 36 (2002) 2783.
[35] USEPA. Office of Research and development, National Center for Environmental Assessment, Washington, DC, 1988.
[36] National Academy of Science, National Research Council of the National Academies Press, Washington, DC, 2005.
[37] M.A. Greer, G. Goodman, R.C. Pleus, S.E. Greer, Environ. Health Perspect. 110 (2002) 927.
[38] C.A. Sanchez, K.S. Crump, R.I. Krieger, N.R. Khandaker, J.P. Gibbs, Environ. Sci. Techol. 39 (2005) 9391.

EXHIBIT D

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: 378–455, 2012)**

ISSN Print 0163-769X ISSN Online 1945-7189
Printed in U.S.A.

doi: 10.1210/er.2011-1050 Received October 27, 2011. Accepted February 7, 2012.
First Published Online March 14, 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 chemical; 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.

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 μg/kg · d (679)	No NOAEL was ever established in toxicological studies (38)	<50 mg/kg · d (38)	~400 μg/kg · d to rodents and nonhuman primates (4, 253)
DEHP	0.5–25 μg/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 μg/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)α and ERβ], 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 α-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) 20–800 pg/ml (premenopausal) <30 pg/ml (postmenopausal)		10–60 pg/ml (adult)
Progesterone		0.2–0.55 ng/ml (prepubertal) 0.02–0.80 ng/ml (follicular phase) 0.90–4 ng/ml (luteal phase) <0.5 ng/ml (postmenopausal)		0.1–0.4 ng/ml (prepubertal) 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.

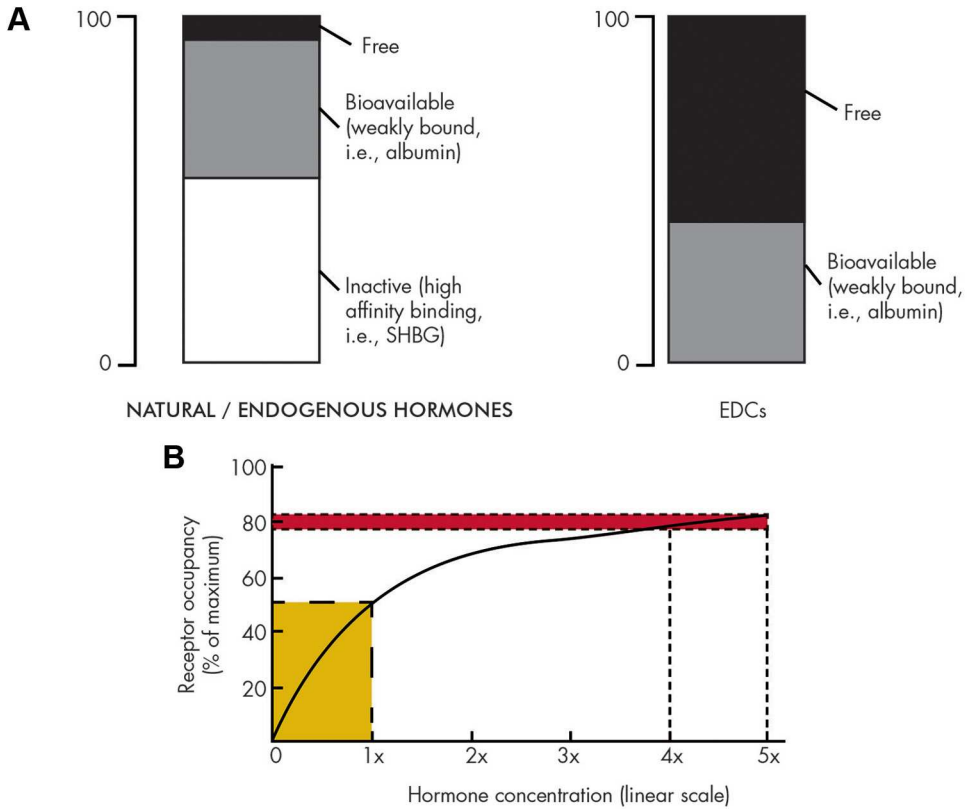


Figure 1. Characteristics and activities of natural hormones. A, This schematic depicts a typical relationship of three phases of circulating hormones: free (the active form of the hormone), bioavailable (bound weakly to proteins such as albumin), and inactive (bound with high affinity to proteins such 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)α and mERβ] 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 ERα and ERβ 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 α-fetoprotein, a plasma protein produced in high levels by the fetal liver (147). Some estrogen-like EDCs, however, bind very weakly to α-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 α-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.

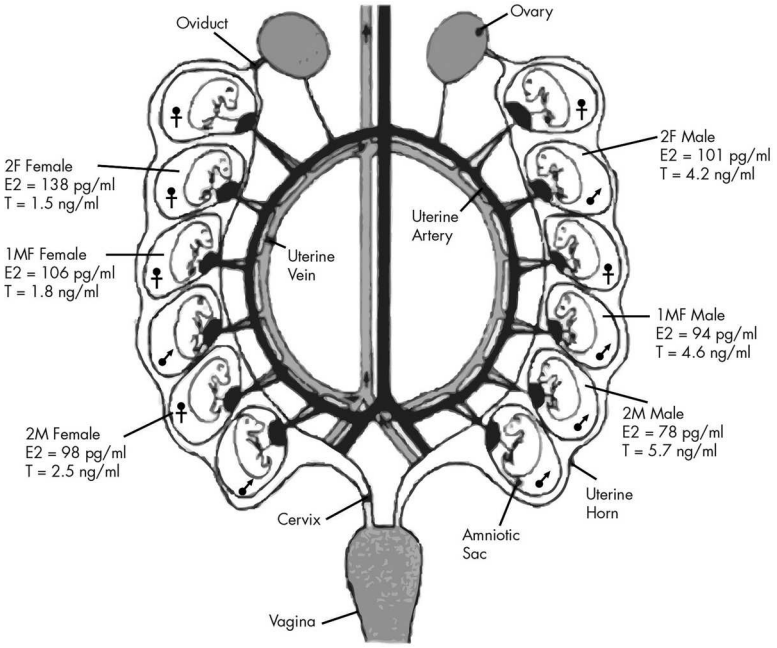


Figure 2. Intrauterine position produces offspring with variable circulating hormone levels. Fetuses are fixed in position in the bicornate rodent uterus, thus delivery via cesarean section has allowed for study of the influence of intrauterine position on behaviors, physiology, and organ morphology. 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 μg/liter (334, 335)	Male sexual differentiation/development	See this review
BPA	Plastics, thermal papers, epoxy resins	Binds ER, mER, ERRγ, PPARγ, may weakly bind TH receptor and AR	400 μg/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 μg/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 μg/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 PPARγ	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 PPARγ	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; PPARγ, peroxisome proliferator-activated receptor-γ; 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 ap-

proaches (*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 EDCs 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 17β-estradiol, is most appropriate. The use of 17β-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-γ 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 μg/kg · d based on a LOAEL of 50 mg/kg · d (38). More recent pharmacokinetic scaling experiments have estimated that exposures to approximately 400 μg/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 μg/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 μg/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 μg/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 17β-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 μg/kg · d; most effects were seen at doses below 50 μg/kg · d. Furthermore, PIN lesions were reported after neonatal exposure to 10 μg/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 ERα compared with 17β-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 μg/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 μg/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 μg/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 μg/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 μg/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 μg/liter (334, 335). Thus, using the definitions of low dose established by the NTP (2), we consider any treatment at or below 200 μg/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 μg/liter throughout larval development (336). All doses from 0.1–200 μg/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 μg/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 (17β-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 μg/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 μg/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 μg/kg · d and set the NOAEL at 1 μg/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 μg/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 μg/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 μg/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 μg/dl T_4 reduction (reference range = 5–12 μg/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 μg/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 μg/kg · d dose required to suppress iodide uptake in the Greer *et al.* (438) study or the 500 μg/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.

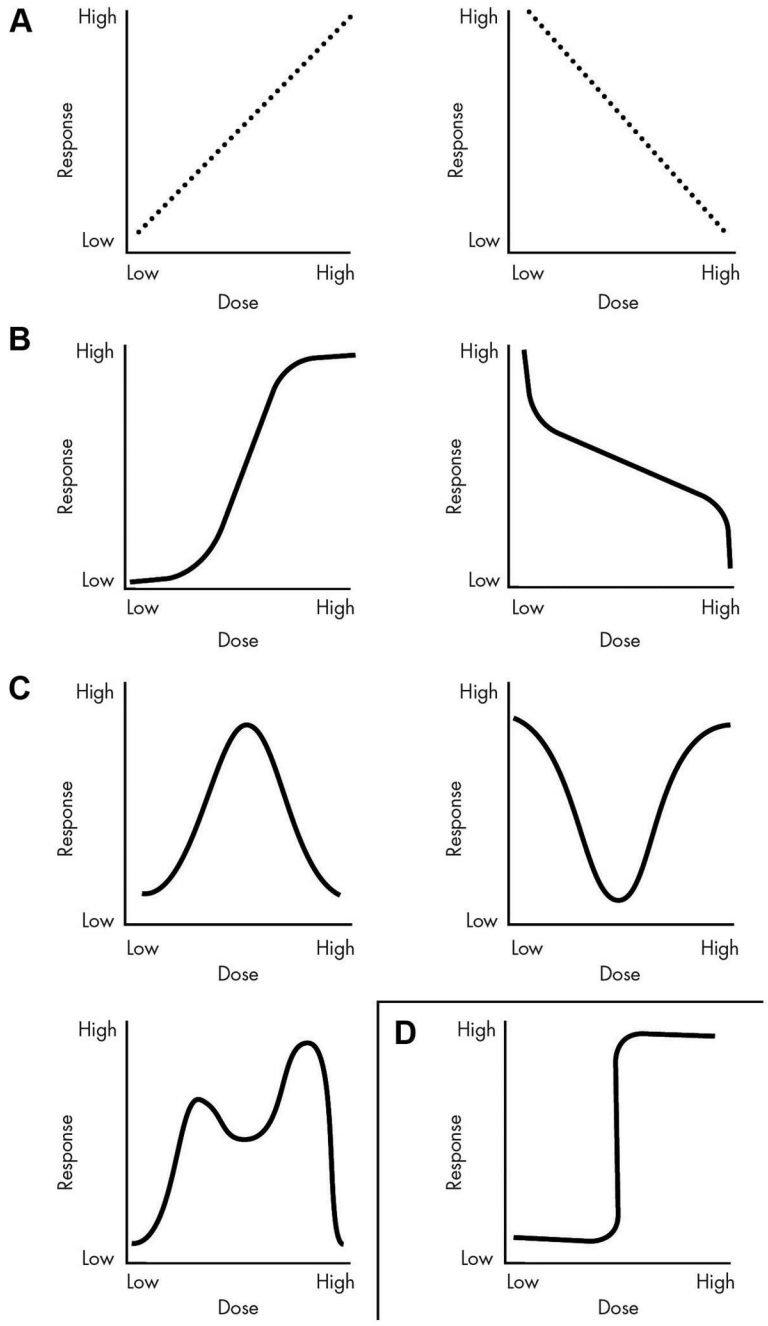


Figure 3. Examples of dose-response curves. A, Linear responses, whether there are positive or inverse associations between dose and effect, allow for extrapolations from one dose to another. Therefore, knowing the effects of a high dose permits accurate predictions of the effects at low doses. B, Examples 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 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.

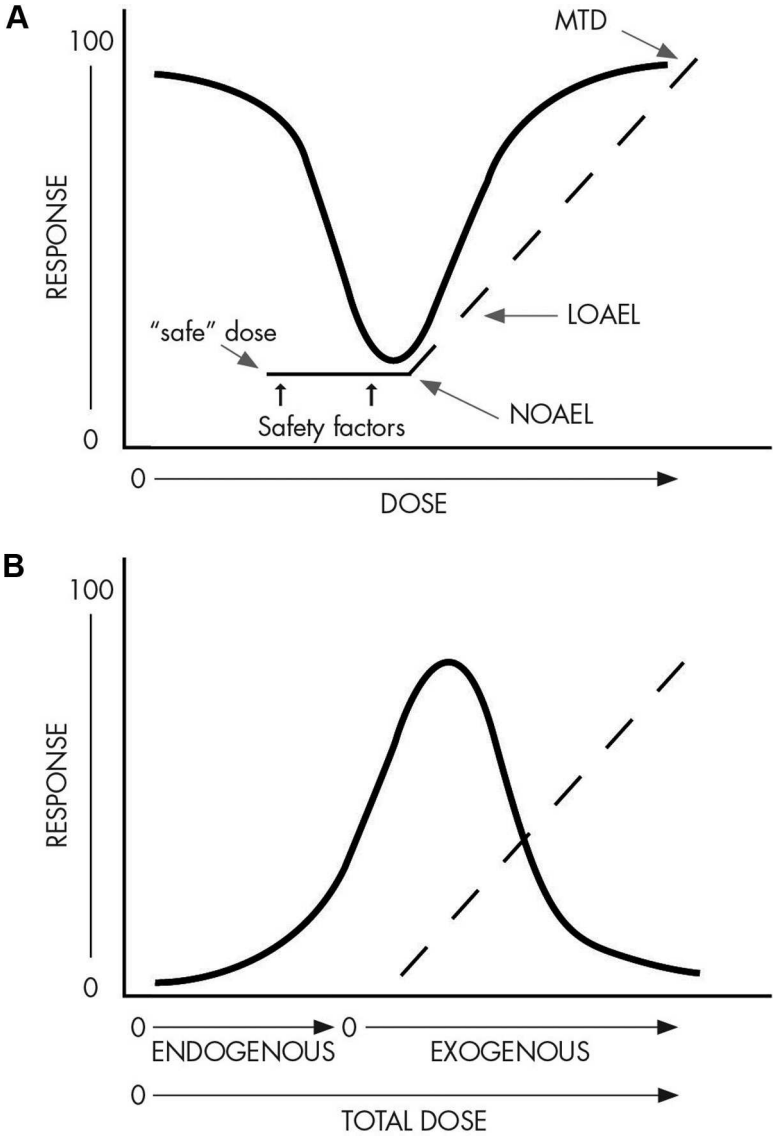


Figure 4. NOAEL, LOAEL, and calculation of a safe reference dose. A, In traditional toxicology testing, high doses are tested to obtain the maximum tolerated dose (MTD), the LOAEL, and the NOAEL. Several safety factors are then applied to derive the reference dose, *i.e.* the dose at which exposures 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 (ERα and ERβ), 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 ERα and ERβ, and the effects of signaling via these two receptors often oppose each other; *i.e.* estrogen action via ERα induces proliferation in the uterus, but ERβ 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)	β-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, ERβ activity	HepG2 liver cells	751
Atrazine	Cell number	IEC-6 intestinal cells	752
Insecticides			
Endosulfan	Cell number	IEC-6 intestinal cells	752
	β-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	β-Hexosaminidase release	HMC-1 mast cells	720
DDT	Cell number	MCF7 breast cancer cells	144
DDE	β-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			
17β-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
11β-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)	26
β-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.

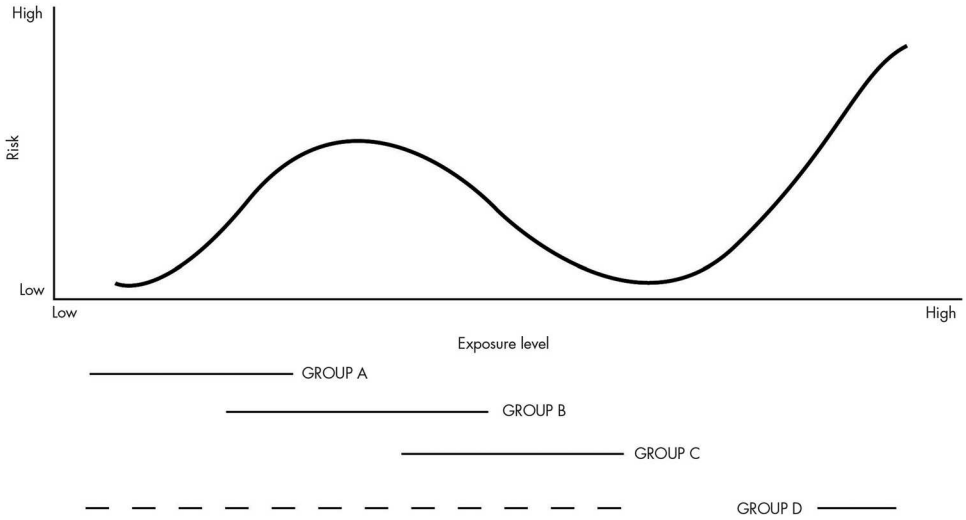


Figure 5. Example of a NMDRC in humans and the sampling populations that could be examined in epidemiology studies. This schematic illustrates a theoretical NMDRC in a human population. If a study were to sample only group A, the conclusion would be that with increasing exposures, risk increases monotonically. 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.

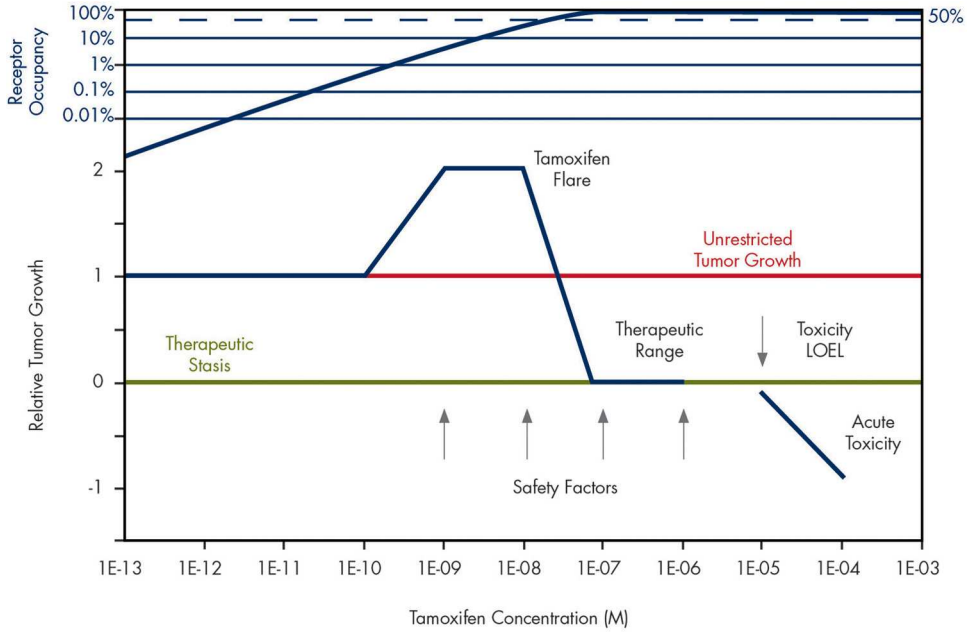


Figure 6. Dose-response ranges for tamoxifen in breast cancer therapy. This figure demonstrates the NMDRC, also called flare, in tamoxifen treatments. As the circulating dose of tamoxifen increases when treatment starts, patients initially experience flare, *i.e.* growth of the tumor (546), followed 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 μM); 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.

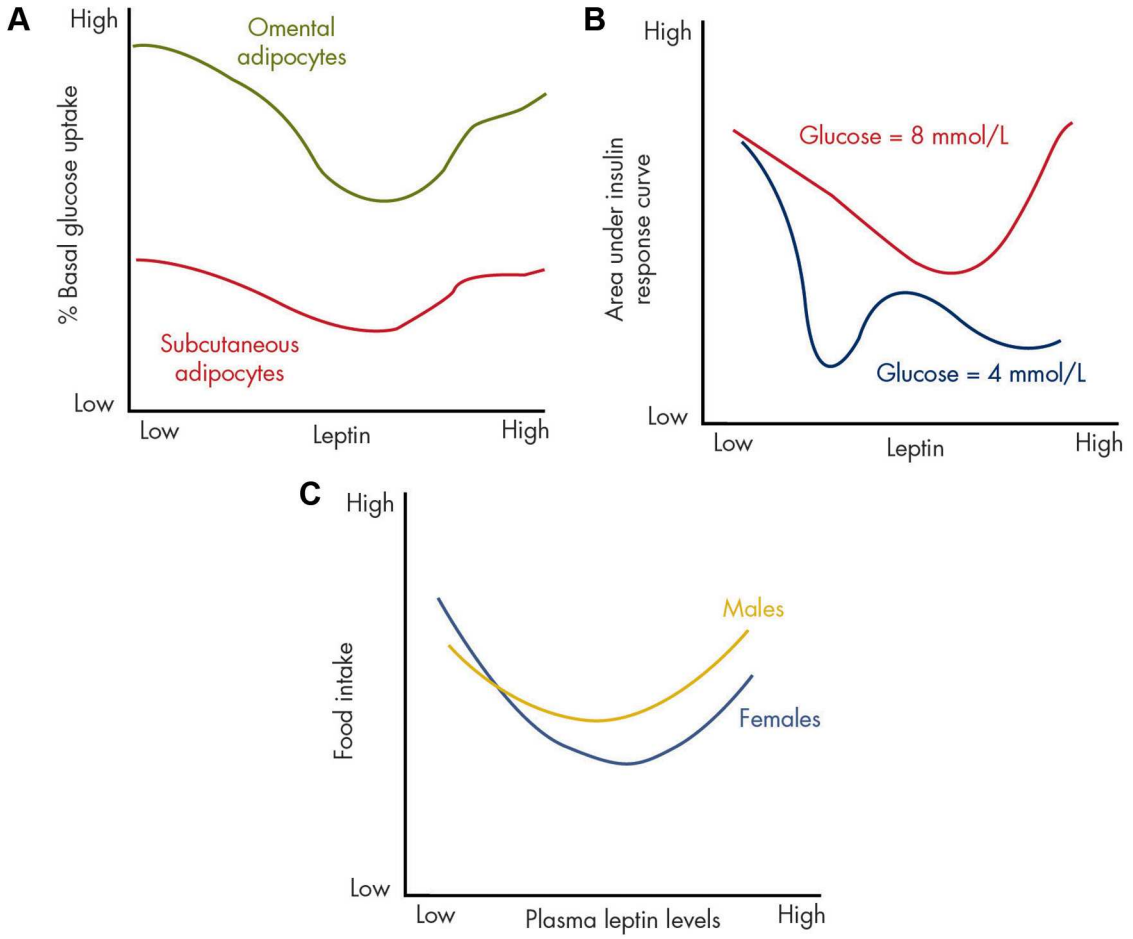


Figure 7. Leptin as an example of a NMDRC. Several studies report NMDRCs in response to leptin treatments. A, NMDRCs are observed in cultured primary adipocytes after leptin exposure. This graph illustrates the relationship between administered leptin dose and glucose uptake in two types of adipocytes, 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 rerelease 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 weight loss, mortality, 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, innervation, 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 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 α-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 17β-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-α 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 β: 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 α-receptor. II. Analysis of the "spare-receptor" hypothesis and estimation of the concentration of α-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-α: 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 α and estrogen receptor β. 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 α and estrogen receptor β. 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 α 1-fetoprotein. J Steroid Biochem 11:237–243
148. **Milligan SR, Khan O, Nash M** 1998 Competitive binding of xenobiotic oestrogens to rat α-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 β 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 17β-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-γ 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 α and β 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 17β-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 TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall Jr LW, Williams M** 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 (17β-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 5α-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 17β-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 downregulation 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 downregulation 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, nonmonotonic 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 α 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 β-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-β 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 α 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-α-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 β 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 GSK3β/β-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 17β-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 Aβ(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 α and β 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 17α-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. **Undeđer 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

EXHIBIT 3

ANDREW C. WILSON
State Bar No. 133062
7468 Dufferin Avenue
Riverside, CA 92504
Telephone: (951) 687-4471
E-mail: acwilson11@yahoo.com

Petitioner In Pro Se

SUPERIOR COURT OF THE STATE OF CALIFORNIA

COUNTY OF LOS ANGELES

CENTRAL DISTRICT

ANDREW C. WILSON, Petitioner. v. **STATE WATER RESOURCES CONTROL BOARD,** Respondent.	Case No. BS149632 **PETITIONER'S OPENING BRIEF** Dept: 85 Judge: The Honorable James C. Chalfant Trial Date: July 28, 2015 1:30 p.m. Action Filed: July 3, 2014

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Cases

Statutes

Regulations

I. INTRODUCTION

Petitioner is an attorney whose principal occupation is farming oranges. Petitioner brings this action against Respondent, a state agency, to set aside an administrative order that declares that treated municipal wastewater, commonly known as "recycled water," is safe for irrigating oranges. In issuing its order, Respondent ignored the risk of harm from a toxic chemical known as "perchlorate."

Perchlorate, a waste product of industrial processes, is particularly dangerous to pregnant women when ingested or swallowed. It adversely affects the development of the unborn baby, resulting in reduced mental capacity of the child.

Perchlorate in irrigation water enters the roots of orange trees and becomes incorporated in the edible portion of the fruit. The concentration level in the fruit is higher than the concentration level in the irrigation water.

The California drinking water safety limit for perchlorate is 6 micrograms per liter. Perchlorate in treated municipal wastewater can be as high as 250 to 700 micrograms per liter.

Respondent's order authorizes treated municipal wastewater to be used for irrigating oranges without testing for perchlorate. Petitioner contested Respondent's order on the ground that the potential for treated wastewater to produce contaminated oranges presents an unreasonable public health risk, and that Respondent's order should require that recycled water be tested for perchlorate prior to use as irrigation water for oranges.

The Respondent held a hearing prior to issuing its order. At the hearing the Respondent received evidence of the risk of perchlorate submitted by Petitioner. Neither Respondent's staff nor anyone else submitted evidence that challenged or conflicted with Petitioner's evidence about perchlorate. In its order, the Respondent made no findings on perchlorate and made no findings that mentioned any of the evidence that Petitioner submitted.

II. STATEMENT OF FACTS

Petitioner ANDREW C. WILSON is an attorney whose principal occupation is farming oranges. (AR 620) Respondent STATE WATER RESOURCES CONTROL BOARD ("State Board") is a state agency and a board of the California Environmental Protection Agency. (Water Code §§ 13001, 13100.) By this action Petitioner seeks to set aside an order adopted by the State Board on June 3, 2014, entitled *General Waste Discharge Requirements for Recycled Water Use*, State Board Water Quality Order 2014-0090 (the "Order"). (AR 1) The State Board adopted the Order pursuant to Water Code § 13263(i). (AR 9)

The State Board developed the Order in response to Governor Brown's drought proclamations of January 17, 2014, and April 25, 2014. (AR 193) The Governor's April 25, 2014, proclamation ordered the State Board to "adopt statewide general waste discharge requirements to facilitate the use of treated wastewater that meets the standards set by the Department of Public Health, in order to reduce the demand on potable water supplies." (AR 397)

The State Board gave public notice that it would hold a meeting to receive public comments on the proposed order. (AR 448) The proposed order stated: "Title 22 imposes limitations on the uses of recycled water, based on the level of treatment and the specific use in order to protect human health. By restricting the use of recycled water to title 22 requirements, *this Order ensures that recycled water is used safely*." (Italics added.) (AR 97)

The State Board received 34 written public comments prior to the hearing. (AR 451-885) Petitioner submitted a written comment that contested the State Board's proposed decision that the Order "ensures that recycled water is used safely" and demonstrated that recycled water should be tested for perchlorate prior to use as irrigation water for oranges.[1] (AR 620-772)

The State Board held a hearing on the Order on June 3, 2014, in Sacramento. Nine persons presented oral comments at the hearing prior to the approval of the Order. (AR 199, 231, 240,

[1] "Perchlorate" refers to an anion (a negatively charged ion) with the formula (ClO_4^-) comprised of a tetrahedral array of 4 oxygen atoms around a central chlorine atom. (AR 640) Perchloric acid and most perchlorate salts will readily dissolve in water, generating the perchlorate anion. (Id.)

270, 273, 279, 283, 293, 300) Petitioner did not appear at the hearing. State Board staff never offered any documents in evidence or made any requests for official notice.

Petitioner submitted four scientific articles as exhibits to his comment letter:

- Greiner, P., et al. (2008) Occurrence of Perchlorate in Sodium Hypochlorite. *Journal of the American Water Works Association* 100(11):68-74 (AR 625-632)
- Massachusetts Department of Environmental Protection (MDEP) (2006) The Occurrence and Sources of Perchlorate in Massachusetts. Draft Report. (AR 633-686)
- Sanchez, C. A., et al., (2006) Potential Perchlorate Exposure From *Citrus* Sp. Irrigated With Contaminated Water. *Analytica Chimica Acta* 567:33-38 (AR 688-693) The co-authors of the Sanchez article included scientists from the University of Arizona, the University of California at Riverside, and the National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. (AR 688)
- Vandenberg, L. N. (2012) Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses. *Endocrine Reviews* 33(3):378-455 (AR 695-772) The co-authors of the Vandenberg article included scientists from Tufts University, the University of California at Berkeley, the University of Minnesota, the University of Missouri-Columbia, the University of Massachusetts-Amherst, Massachusetts General Hospital Center for Cancer Research, the National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, Research Triangle Park, North Carolina, and other institutions. (AR 695)

These articles and Petitioner's comment letter were the only evidence regarding perchlorate that the State Board received. No conflicting evidence was received.

The evidence showed the risk of irrigating oranges with recycled water without testing for perchlorate. Perchlorate reduces "the functioning of the thyroid gland, and poor thyroid function is an important cause of developmental deficits and adult disease." (Vandenberg) (AR 719) In humans, the thyroid gland needs iodide to produce thyroid hormone. (Id.) A compound known as NIS is responsible for transporting iodide into the thyroid gland. Perchlorate inhibits the

ability of NIS to take up iodide. (Id.) The reduced transport of iodide suppresses the production of thyroid hormone. (Id.)

"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. Moreover, it has become clear that even small deficits in circulating thyroid hormone in pregnant women or neonates 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." (Vandenberg, references omitted.) (AR 719)

"There is concern that these perchlorate-contaminated waters may represent a health risk both as sources of drinking water and *irrigation water for food crops*." (Sanchez, italics added.) (AR 688) Human exposures to perchlorate "are likely attributed to both contaminated drinking water and food: in fact, a recent analysis concludes that a majority of human exposure to perchlorate comes from food." (Vandenberg) (AR 719)

Perchlorate is "chemically stable when wet and persists for long periods in geological systems and in ground water." (Vandenberg) (AR 719)

"Studies have shown that perchlorate is not physically or chemically retained by soil. Thus, perchlorate is largely transported into and through soils with irrigation water and the perchlorate concentration of this water is the most reliable estimate of plant available perchlorate over a growing season." (Sanchez) (AR 690)

Orange trees take up perchlorate with irrigation water, and the concentration in the fruit is higher than the concentration in the irrigation water: The Sanchez data showed that orange trees in Loma Linda, California, irrigated with contaminated well water with a perchlorate level of 18 ppb, produced oranges with a perchlorate level of 38 ppb. (AR 690, 692) The California drinking water safety limit for perchlorate is 6 ppb.[2] (AR 620, 627)

[2] The abbreviation "ppb" means "parts per billion." It is similar to per cent, except the expression is "per billion" rather than "per hundred." Thus, 50% is the same as 500,000,000 ppb. A statement that a certain material has a perchlorate concentration of 18 ppb can be conceptualized as meaning that 1 billion pounds of the material contains 18 pounds of perchlorate. Concentrations expressed in ppb are equivalent to concentrations expressed as micrograms per kilogram (µg/kg) or micrograms per liter (µg/l) (for water solutions). (AR 720)

As water evaporates from a tree into the air, salts that have been taken up in the tree with the irrigation water are left behind and accumulate in the tree. (622) Accordingly, salts in plant tissues accumulate to levels that are higher than in the irrigation water. (622)

Treated municipal wastewater can have perchlorate concentrations ranging from 250 ppb to 700 ppb. (AR 678) Even if oranges did not accumulate perchlorate, these levels in fruit would exceed recommended safety levels.[3]

Sanchez assessed perchlorate risk using a benchmark recommended by the National Academy of Sciences of 0.7 micrograms per kilogram of body weight per day (0.7 µg/kg · d), representing the cut-off above which effects begin to occur (referred to as the "no-effect reference dose"). (AR 692) Vandenberg presents data suggesting that lower amounts affect thyroid function in adult women: "The NHANES dataset suggests that perchlorate exposures of 0.2 to 0.4 µg/kg · d are associated with depressed thyroid function." (AR 720)

The Sanchez data showed significantly higher perchlorate concentrations in the orange leaves than the fruit reflecting greater water transpiration through leaves. (AR 692) The average fruit concentration was 7.4 ppb and the average leaf concentration was 1,424 ppb. (AR 692) Sanchez noted perchlorate accumulation in leafy green vegetables. (AR 688) Vandenberg stated that "both aquatic and terrestrial plants can concentrate perchlorate more than 100-fold over water levels." (AR 719)

---

[3] Sanchez estimated the perchlorate exposure from eating oranges with a perchlorate level of 4.8 ppb, which was the median perchlorate level of all the oranges sampled in the study, and concluded that the potential exposure from 4.8 ppb in the edible fruit is small relative to the no effect reference dose recommended by the National Academy of Sciences. (AR 692) The other irrigation sources included in the Sanchez study had much lower perchlorate levels than the Loma Linda well; many had no detectable perchlorate. (AR 691) Sanchez assessed the risk of exposure using estimates of average daily fresh orange consumption for adults (.249 kg/day) and children (.107 kg/day). (AR 692) For example, the daily exposure for an adult from fruit that had a perchlorate level of 250 µg/kg would be 62.3 µg (.249 x 250 = 62.3). Thus, for a 70 kg adult, the daily exposure would be 0.89 micrograms per kilogram of body weight (62.3 ÷ 70 = 0.89), which is abbreviated 0.89 µg/kg bw. A daily exposure of 0.89 µg/kg bw exceeds the no effect reference dose of 0.7 µg/kg bw recommended by the National Academy of Sciences. (AR 692) For a 10 kg child, the daily exposure would be 2.7 µg/kg bw, almost 4 times the recommended no effect reference dose. (AR 692)

Perchlorate can be introduced into municipal sewers as waste discharged from industrial processes that use perchloric acid. (AR 676-678) According to the Massachusetts Department of Environmental Protection (MDEP) Draft Report:

> "Perchloric acid has the same unique and desirable properties as perchlorate salts: a powerful oxidizing agent that is at the same time safe to use. While the extent of its use in Massachusetts is not at the present known, *it is clear that industrial-scale discharges of process wastewaters containing this material has the potential to create significant impacts to groundwater and surface water.*" (Italics added.) (AR 676)[4]

In 2004 the MDEP investigated perchlorate contamination in the Merrimack River, the second largest river in Massachusetts. (AR 677) The contamination was traced upstream to a community wastewater treatment plant. (Id.) The effluent from the treatment plant showed consistent levels of perchlorate in the range of 250 ppb to 700 ppb. (AR 678) The treatment plant system was a secondary treatment system that served a community of 50,000. (Id.) Investigations identified the source of perchlorate to be rinse water discharged to the sewer by a processor of surgical and medical materials. (Id.) The company used approximately 220 gallons of perchloric acid per month. (Id.) The company subsequently used ion-exchange technology to reduce perchlorate levels in the company's discharge to below 50 ppb. (Id.)

III. REGULATORY SUMMARY

The State Board consists of five members representing the state at large that the Governor appoints for 4 year terms. (Water Code §§ 175, 177.) The Legislature has divided the state into nine regions, and for each region has established a Regional Water Quality Control Board ("Regional Board"). (Water Code §§ 13200, 13201.) Each Regional Board consists of nine members the Governor appoints for 4 year terms. (Water Code §§ 13201, 13202.)

---

[4] "Although a strong oxidizing agent, the perchlorate anion is persistent in the environment, due to the high activation energy associated with its (abiotic) reduction to chlorate (ClO_3). Moreover, given its low charge density, perchlorate does not form complexes with metals in the same manner as other anions, and, in its ionic state, does not readily sorb to environmental media." (AR 640)

The Porter-Cologne Water Quality Control Act (the "Act") protects the quality of the waters of the state, including both surface water and ground water, through a permitting process that controls the discharge of "waste."[5]

The Act requires persons to report proposed discharges of waste that could adversely affect the quality of the waters of the state to their local Regional Board. (Water Code §§ 13260.) Regional Boards may require testing to assess the safety of the discharge. (Water Code § 13267.) Regional Boards issue permits called "waste discharge requirements." (Water Code §§ 13263.) As an example, a factory must obtain a permit from its local Regional Board to discharge waste into a river. A rainstorm may wash chemicals from the factory site into the river, and that storm water will be considered a discharge of waste requiring a permit. The factory may pond wastewater on site, causing wastewater to percolate down through the soil to the ground water and contaminate the drinking water wells of neighboring homeowners. That also will be considered a discharge requiring a permit. Permits must prevent nuisance, which includes anything that is injurious to public health. (Water Code §§ 13263(a), 13050(m).)

The Act authorizes two types of waste discharge requirements -- "individual" and "general." (Water Code § 13263(i)(4).) General waste discharge requirements cover a category of discharges and a large number of permittees. For example, piles of dirt at construction sites are prone to be washed into streets, drains and adjoining properties during rainstorms. General waste discharge requirements may be issued requiring that remedial measures be taken at construction sites, such as perimeter sandbagging. Contractors then "enroll" in the general permit rather than obtaining an individual permit. State Board general permits apply statewide. Regional Board general permits apply only in the specific region.

Chapter 7 of the Act establishes reporting and permit requirements for the use of recycled water and water recycling facilities. The Act defines recycled water: "'Recycled water' means

[5] The Act is set forth in Water Code §§ 13000 et seq. The Act defines "waste" as follows: "'Waste' includes sewage and any and all other waste substances, liquid, solid, gaseous, or radioactive, associated with human habitation, or of human or animal origin, or from any producing, manufacturing, or processing operation, including waste placed within containers of whatever nature prior to, and for purposes of, disposal." (Water Code § 13050(d).)

water, which, as a result of treatment of waste, is suitable for a direct beneficial use or a controlled use that would not otherwise occur and is therefore considered a valuable resource." (Water Code § 13050(n).) The Act requires persons to report the use of recycled water in advance to their local Region Board. (Water Code §§ 13522.5.) Regional Boards may require testing to assess the safety of the recycled water. (Id.) Regional Boards issue two types of permits authorizing the use of recycled water: "water recycling requirements" and "master recycling permits." (Water Code §§ 13523, 13523.1) Master recycling permits are intended to cover a large number of users and are issued to suppliers or distributors rather than users.

The California Department of Public Health has established statewide recycling criteria in California Code of Regulations, title 22, division 4, chapter 3 ("title 22"). Title 22 requires that recycled water receive certain levels of treatment, depending on its proposed use.

In a toilet bowl tiny particles of fecal material may be suspended in the water giving the water a cloudy appearance. The presence of suspended solid particles in wastewater is the meaning of "turbidity" used in title 22. Primary stage treatment involves allowing solids to settle to the bottom, which helps reduce turbidity. "Secondary" treatment means oxidation – bubbling air or oxygen through the water to create dissolved oxygen. Oxidation promotes the activity of micro-organisms that break down organic matter, which helps reduce odors. "Tertiary" treatment means filtering the water to reduce turbidity to certain standards.

Title 22 provides that recycled water shall be at least "undisinfected secondary recycled water" when used for surface irrigation of orchards where the recycled water does not come in contact with the edible portion of the crop. (Title 22, § 60304(d)(1).) "[U]ndisinfected secondary recycled water" simply means oxidized wastewater. (Title 22, § 60301.900.)

For crop irrigation, title 22's highest standard is "disinfected tertiary recycled water," which applies when recycled water is used for the surface irrigation of food crops where the recycled water comes into contact with the edible portion of the crop. (Title 22, § 60304(a)(1).) "[D]isinfected tertiary recycled water" means wastewater has been filtered and subsequently disinfected. (Title 22, § 60301.230.) To be considered "filtered," the wastewater must be oxidized and filtered to meet specific turbidity standards. (Title 22, § 60301.320.) Disinfection

means reducing organisms that cause disease, or "pathogenic" organisms.[6] Title 22 does not dictate the method of disinfection, but allows various methods, including adding sodium hypochlorite to the water. Sodium hypochlorite is the active ingredient in household bleach.[7]

IV. THE ORDER

The Order in this case constitutes general waste discharge requirements for recycled water use issued under Water Code § 13263(i). (AR 1, 9). To obtain coverage (enroll) under the Order, an applicant submits a "Notice of Intent" to the local Regional Board. (AR 16) The applicant cannot proceed until the Regional Board gives its approval, which is a ministerial act performed by the Executive Officer with no requirement of a public hearing.[8] (AR 16, 19)

V. JURISDICTION

This Court has jurisdiction over this Petition pursuant to Code of Civil Procedure § 1094.5. Section 1094.5 governs review of a State Board "order," including "a final action in an adjudicative proceeding and an action subject to Section 11352 of the Government Code." (Water Code § 13330(g).) The Order in this case is both. Actions subject to Section 11352 of the Government Code include:

> "The issuance, denial, or revocation of *waste discharge requirements and permits pursuant to Sections 13263 and 13377 of the Water Code* and waivers issued pursuant to Section 13269 of the Water Code." (Gov. Code § 11352(b), italics added.)

---

[6] To be considered "disinfected," within the meaning of disinfected tertiary recycled water, the wastewater must meet both (a) and (b):

(a) (1) The wastewater must be subjected to a chlorine disinfection process for a certain specific time following filtration, or
(2) Subjected to a disinfection process that, when combined with filtration, has been demonstrated to inactivate certain viruses to a specific standard.

(b) After the disinfection process the number of surviving so-called "coliform" bacteria is below certain specific limits. (Title 22, § 60301.230.)

[7] The concentration of sodium hypochlorite in commercial solutions ranges from about 6% (by weight) in household bleach, to up to about 16% (by weight) in solutions used at wastewater treatment facilities. (AR 670)

[8] The hearing transcript shows that Regional Board approval is a ministerial act without a public hearing. (See AR 253-254 (an exchange between the Chair of the State Board and Mr. Regan, Senior Staff Counsel to the State Board), see also AR 314 (an exchange between the Chair of the State Board and Mr. Bishop, Chief Deputy Director of the State Board.)

The above-quoted language refers to "waste discharge requirements" issued pursuant to Section 13263 without distinguishing between "individual" or "general" waste discharge requirements. Both types are issued pursuant to Section 13263 (and Section 13377). Since the language of Government Code Section 11352 does not make any distinction, or carve out any sub-category of waste discharge requirements, the language is properly construed to include both types of waste discharge requirements. [9]

VI. STANDING

Petitioner has public interest standing to bring this action. Where the question is one of public right and the object of mandamus is to procure the enforcement of a public duty, the petitioner need not show any legal or special interest in the result, because it is sufficient that the petitioner is interested as a citizen in having laws executed and the duty in question enforced. (*Rialto Citizens For Responsible Growth v. City of Rialto* (2012) 208 Cal.App.4th 899, 914 [146 Cal.Rptr.3d 12]; *Diaz v. Quitoriano* (1969) 268 Cal.App.2d 807, 811-812 [74 Cal.Rptr. 358].)

The enforcement of a duty is a matter of public right when it involves a matter of "statewide" concern. (*Diaz, supra,* 268 Cal.App.2d at p. 811; *Board of Social Welfare v. County of Los Angeles* (1945) 27 Cal.2d 98, 100-101 [162 P.2d 627].) The State Board administers a statewide program of water quality control for the benefit of all the people in the state. The State Board in its Answer admits that "the control of recycled water quality to protect human health is a mater of statewide concern." (Answer, p. 3; *see also* Water Code § 13000; *Hampson v. Superior Court* (1977) 67 Cal.App.3d 472, 484 [136 Cal.Rptr. 722].) The proper performance by the State Board of its statutory duties under the statewide program is a matter of public right. (*Diaz, supra,* 268 Cal.App.2d at p. 811.)

Petitioner also has standing because as a farmer, he has an interest in the Order over and above the interest held in common with the public at large. Water agencies and suppliers throughout California can rely on and use the Order in efforts to convert all farmers in the state to recycled water use. The class of persons that may become directly subject to the terms of the

[9] Since Section 1094.5 applies to general waste discharge requirements, Chapter 4.5 of the Administrative Procedure Act also applies. (Gov. Code § 11410.10.)

Order include "Producers, Distributors, and Users." (AR 12) As a California farmer, Petitioner is a member of this class.

VII. STANDARD OF REVIEW

The court "review[s] questions of law de novo." (*Duncan v. Department of Personnel Admin.* (2000) 77 Cal.App.4th 1166, 1174 [92 Cal.Rptr.2d 257].) This case involves no fundamental vested rights. Findings of fact are reviewed under the substantial evidence standard. (*Bixby v. Pierno* (1971) 4 Cal.3d 130, 144 [93 Cal.Rptr. 234].)

VIII. ARGUMENT

A. The Order Is Not Supported By The Findings.

The California Supreme Court has held: "We further conclude that implicit in section 1094.5 is a requirement that the agency which renders the challenged decision must set forth findings to bridge the analytical gap between the raw evidence and ultimate decision or order." (*Topanga Assn. for a Scenic Community v. County of Los Angeles* (1974) 11 Cal.3d 506, 515 [113 Cal.Rptr. 836]; Gov. Code § 11425.10 (a)(6).)

The Order fails to set forth findings that bridge the analytical gap between the raw evidence on perchlorate and the decision that: "By restricting the use of recycled water to title 22 requirements, this Order ensures that recycled water is used safely." (AR 13)

The Order contains no findings on perchlorate -- it never mentions perchlorate at all. With a complete lack of findings, it is impossible to know why the State Board decided that title 22 ensures that perchlorate levels are safe for citrus.

Proper findings demonstrate that the decision rests solely on the legal rules and evidence adduced at the hearing. (*Goldberg v. Kelly* (1970) 397 U.S. 254, 271 [25 L.Ed.2d 287, 90 S.Ct. 1011].) Inadequate findings leave room for the possibility of an erroneous determination. (*Los Alamitos Gen. Hosp. Inc. v. Lackner* (1978) 86 Cal.App.3d 417, 425 [149 Cal.Rptr. 98].) Petitioner believes that Respondent would agree that the following hypothetical determinations are all erroneous. These are hypothetical examples only; they do not appear in the Order:

- *Title 22 sets forth numeric limits for perchlorate in recycled water that are safe for citrus.* The recycled water provisions of title 22 do not mention perchlorate.

- *Title 22 requires recycled water to be disinfected. The disinfection process will remove any perchlorate.* A commonly used disinfectant is sodium hypochlorite. (AR 625, 675) The addition of sodium hypochlorite will not remove perchlorate, if anything, it will add perchlorate. (AR 630-631, 675)
- *Title 22 requires that the turbidity of the wastewater be reduced. Reducing turbidity will reduce perchlorate to safe limits.* In water perchlorate is not in suspension, it is a dissolved anion. (AR 640)
- *Petitioner's scientific articles were not given any weight because the articles have been discredited by subsequent research.* Petitioner is aware of no such research.

It is impossible to know the basis for the State Board's decision. It is a matter of speculation. Speculation, however, is not permitted. There is "no room for the conclusion that the Legislature would have been content to have a reviewing court speculate as to the administrative agency's basis for decision." (*Topanga, supra*, 11 Cal.3d at p. 515.)

B. <u>The Findings Are Not Supported By Substantial Evidence</u>.

Petitioner contests the decision that "[b]y restricting the use of recycled water to title 22 requirements, this Order ensures that recycled water is used safely." If this is considered to be a finding, it is not supported by substantial evidence.[10]

State Board staff introduced no evidence regarding perchlorate. No one mentioned perchlorate at the hearing. Title 22 does not mention perchlorate. State Board staff introduced no evidence that the purpose or effect of the title 22 criteria is to reduce or remove perchlorate from municipal wastewater.

[10] Substantial evidence has been defined in two ways: (1) as evidence of ponderable legal significance, reasonable in nature, credible, and of solid value, and (2) as relevant evidence that a reasonable mind might accept as adequate to support a conclusion. (*County of San Diego v. Assessment Appeals Bd. No. 2* (1983) 148 Cal.App.3d 548, 555 [195 Cal.Rptr. 895].) An abuse of discretion is established if findings are not supported by substantial evidence in light of the whole record. The substantial evidence standard requires the reviewing court to consider all relevant evidence in the record, including evidence that fairly detracts from the evidence supporting the agency's decision. (*County of San Diego, supra*, 148 Cal.App.3d at p. 555; *Bixby, supra*, 4 Cal. 3d at 130 n.22.) Petitioner has the burden of demonstrating that the findings are not supported by substantial evidence. A party challenging the sufficiency of the evidence must summarize and cite to all material evidence, not just evidence favorable to the challenging party.

The record contains no evidence that conflicts with the evidence that Petitioner presented about perchlorate. That evidence showed that municipal wastewater can have perchlorate levels measuring from 250 to 700 ppb. The evidence showed that the perchlorate level in oranges is higher than the level in the irrigation water. The evidence showed that 250 ppb in oranges results in estimated perchlorate exposures in excess of the reference dose recommended by the National Academy of Sciences for adults and children. The evidence showed that there exists data suggesting that the reference dose recommended by the National Academy of Sciences is not protective of adult women.

In light of all the evidence, a reasonable person would take steps to prevent serious injury to an unborn child. A reasonable person would not use recycled water to irrigate oranges if the water has not been tested for perchlorate. A reasonable person would recognize the need for further investigation. The Order approves conduct, the use of un-tested recycled water, that creates an unreasonable risk to public health.

The lack of supporting evidence, together with the detracting evidence presented by Petitioner, establishes that there is not substantial evidence supporting a finding that this "Order ensures that recycled water is used safely."

IX. CONCLUSION

For all the foregoing reasons Petitioner respectfully requests that the Order be set aside.

Respectfully submitted,

Dated: March 30, 2015

ANDREW C. WILSON

Andrew C. Wilson

Andrew C. Wilson
Petitioner In pro se

POS-030

ATTORNEY OR PARTY WITHOUT ATTORNEY (Name, State Bar number, and address):	FOR COURT USE ONLY
ANDREW C. WILSON STATE BAR NO. 133062 7468 Dufferin Avenue Riverside, CA 92504 TELEPHONE NO. (951) 687-4471 FAX NO. (Optional): E-MAIL ADDRESS (Optional): acwilson11@yahoo.com ATTORNEY FOR (Name): In pro se	
SUPERIOR COURT OF CALIFORNIA, COUNTY OF Los Angeles STREET ADDRESS: 111 North Hill Street MAILING ADDRESS: CITY AND ZIP CODE: Los Angeles, CA 90012 BRANCH NAME: Central District - Stanley Mosk Courthouse	
PETITIONER/PLAINTIFF: ANDREW C. WILSON RESPONDENT/DEFENDANT: STATE WATER RESOURCES CONTROL BOARD	
PROOF OF SERVICE BY FIRST-CLASS MAIL—CIVIL	CASE NUMBER: BS149632

(Do not use this Proof of Service to show service of a Summons and Complaint.)

1. I am over 18 years of age and **not a party to this action.** I am a resident of or employed in the county where the mailing took place.

2. My residence or business address is:
   7468 Dufferin Avenue
   Riverside, CA 92504

3. On *(date)*: March 30, 2015 I mailed from *(city and state)*: Riverside, California
   the following **documents** *(specify)*:
   Petitioner's Opening Brief

   ☐ The documents are listed in the *Attachment to Proof of Service by First-Class Mail—Civil (Documents Served)* (form POS-030(D)).

4. I served the documents by enclosing them in an envelope and *(check one)*:
   a. ☑ **depositing** the sealed envelope with the United States Postal Service with the postage fully prepaid.
   b. ☐ **placing** the envelope for collection and mailing following our ordinary business practices. I am readily familiar with this business's practice for collecting and processing correspondence for mailing. On the same day that correspondence is placed for collection and mailing, it is deposited in the ordinary course of business with the United States Postal Service in a sealed envelope with postage fully prepaid.

5. The envelope was addressed and mailed as follows:
   a. **Name** of person served: Eric M. Katz, Supervising Deputy Attorney General
   b. **Address** of person served:
      300 South Spring Street, Suite 1702
      Los Angeles, CA 90013

   ☐ The name and address of each person to whom I mailed the documents is listed in the *Attachment to Proof of Service by First-Class Mail—Civil (Persons Served)* (POS-030(P)).

I declare under penalty of perjury under the laws of the State of California that the foregoing is true and correct.

Date: March 30, 2015

Margaret C. Wilson
(TYPE OR PRINT NAME OF PERSON COMPLETING THIS FORM)

▶ Margaret C Wilson
(SIGNATURE OF PERSON COMPLETING THIS FORM)

EXHIBIT 4

KAMALA D. HARRIS
Attorney General of California
ERIC M. KATZ
Supervising Deputy Attorney General
State Bar No. 204011
CAROL A. Z. BOYD
Deputy Attorney General
State Bar No. 165988
300 South Spring Street, Suite 1702
Los Angeles, CA 90013
Telephone: (213) 897-2630
Fax: (213) 897-2802
E-mail: Eric.Katz@doj.ca.gov
E-mail: Carol.Boyd@doj.ca.gov
Attorneys for Respondent
State Water Resources Control Board

[EXEMPT FROM FILING FEES – GOV. CODE SECTION 6103]

SUPERIOR COURT OF THE STATE OF CALIFORNIA

COUNTY OF LOS ANGELES

CENTRAL BRANCH

ANDREW C. WILSON, Petitioner, **v.** **STATE WATER RESOURCES CONTROL BOARD,** Respondent.	Case No. BS149632 **RESPONDENT'S OPPOSITION BRIEF** Action Filed: July 3, 2014 Trial Date: July 28, 2015 Time: 1:30 p.m. Dept: 85 Judge: Hon. James C. Chalfant

TABLE OF AUTHORITIES

Page

CASES

STATUTES

TABLE OF AUTHORITIES
(continued)

Page

TABLE OF AUTHORITIES
(continued)

Page

OTHER AUTHORITIES

INTRODUCTION

15 years ago the California Department of Public Health (CDPH) determined that using recycled water to irrigate citrus and other crops was safe for human health. (Cal Code Regs., tit. 22, § 60304.) In 2014, in response to the crippling drought and the strain it was placing on potable water sources, the Governor directed respondent State Water Resources Control Board (State Water Board) to take action to streamline the permitting of new uses of recycled water. Specifically, the Governor directed the State Water Board to adopt a statewide "general" permit for the discharge of recycled water, as an alternative to the existing method of permitting recycled water uses on a facility-by-facility and region-by-region basis. In conformance with the Governor's executive order, on June 3, 2014, the State Water Board adopted General Waste Discharge Requirements for Recycled Water Use (General WDRs), which is a generic permit that recycled water producers and users can enroll in and serves as a permit for certain uses of recycled water on the terms provided.

Despite Petitioner's claims to the contrary, the State Water Board's findings comply with *Topanga* because they adequately disclose "the analytic route the administrative agency traveled from evidence to action." (*Topanga Assn. for a Scenic Community v. County of Los Angeles* (1974) 11 Cal.3d 506, 515.) Assuming that the State Water Board was required to make findings with respect to recycled water's potential human health impacts, the State Water Board explicitly made a finding that the use of recycled water in accordance with all regulatory requirements is safe from a human health perspective. (AR 6-7 ¶¶ 9-10.) The State Water Board's finding with respect to human health was based on CDPH's long standing prior regulatory determination that it is safe to human health to irrigate citrus crops with recycled water. (Cal. Code Regs., tit. 22, § 60304, subd. (d).) CDPH's prior regulatory determination is substantial evidence to support to the State Water Board's finding. The Legislature specifically tasked CDPH to adopt regulations governing the uses of recycled water to protect human health (Wat. Code, §§ 13520, 13521), and the State Water Board reasonably relied on its sister agency's human health determination.

The analytical route the State Water Board traveled is clear: The State Water Board found that use of recycled water consistent with CDPH's regulatory requirements is safe for human

health, and that finding is supported by substantial evidence. The petition for writ of mandate should be denied.

BACKGROUND

I. THE CONTINUALLY EXPANDED USE OF RECYCLED WATER IS A KEY ELEMENT OF CALIFORNIA'S WATER POLICY

In 1969, the Legislature adopted the Water Recycling Law,[1] declaring that the people of the state have a primary interest in the development of recycled water facilities to supplement existing water supplies. (Wat. Code, §§ 13500, 13501 (West. 1971).) The Legislature further found that the use of recycled water for agricultural uses will contribute to the "peace, health, safety and welfare" of the people of the state. (*Id.* § 13511 (West. 1971).)

Over the years, the Legislature has continued to express its intent that the use of recycled water be increased. For example, in 1977, the Legislature mandated that recycled water be used for certain applications, such as irrigating cemeteries, golf courses, and parks, if certain conditions are met. (Wat. Code, § 13550.) In 1983, the Legislature required that local government's urban water management plans contain an analysis of the potential for recycled water to be used for irrigated agriculture. (Wat. Code, § 10633, subd. (d).) In 1991, the Legislature established a statewide goal to recycle 700,000 acre feet of water per year by 2000, and 1 million acre feet of water per year by 2010. (Wat. Code, § 13577.) In 2009, the State Water Board declared that it is "waste" to use potable water when recycled water of adequate quality is available; the California Constitution prohibits the waste of water. (AR 408; Cal. Const., art. X, § 2.) In 2010, the State Water Board adopted an increased goal of recycling at least 2 million acre feet[2] of recycled water per year by 2030. (Wat. Code, § 13560, subd. (a).) In

---

[1] The original act was known as the Water *Reclamation* Act. (Wat. Code, § 13500 (West. 1970), italics added.) By a 1996 amendment, the name of the act was changed to the Water *Recycling* Act, and all uses of the term "reclaimed" water were substituted with the term "recycled" water. (Wat. Code, § 13500 (West. 1995); Stats. 1995, c. 28 (A.B. 1247).) To keep with contemporary parlance and for ease of reading, this brief will use the term recycled water even when referring to pre-1995 statutes that used the term reclaimed water.

[2] By way of comparison, 2 million acre feet of water is enough to supply about 4 million households with domestic water for one year. The entire State Water Project, in an average water year, delivers about 2.4 million acre feet of water. (RJN Ex. C [DWR State Water Project Delivery Reliability Report 2013, at p. 35 at Table 4-4].) Recycled water, therefore, is expected to supply almost as much water as the entire State Water Project by 2030.

2010, the Legislature directed CDPH to adopt regulations governing the indirect potable use of recycled water for groundwater recharge. (Wat. Code, § 13562.) The Governor's California Water Action Plan, released in 2014, specifically calls for the increased use of recycled water as a key step to meet the state's overall water policy goal of increasing regional self-reliance. (Request for Judicial Notice (RJN), Ex. A [Water Action Plan at p. 7].) In sum, the State has maintained a consistent policy to continually increase the use of recycled water in the state.

II. THE STATE'S REGULATION OF RECYCLED WATER

At all relevant times,[3] two state agencies have responsibilities with respect to regulating recycled water. CDPH, not a party to this lawsuit, has primary responsibility for setting standards for the use of recycled water for the protection of public health. The State Water Board, and the nine regional water quality control boards, have primary responsibility for regulating recycled water for the protection of water quality. In 1996, the two agencies executed a Memorandum of Agreement delineating their respective obligations. (AR 10; RJN Ex. B [1996 MOA].)

A. CDPH's Regulation of Recycled Water

CDPH is the primary state agency responsible for protection of public health. (AR 6, 10; RJN Ex. B [1996 MOA at p. 2].) Among its public health responsibilities is the regulation of recycled water's impacts on public health. (Wat. Code, § 13520, 13521.) The Legislature specifically empowered CDPH to establish statewide recycling criteria for different types of uses of recycled water for the protection of public health. (*Id.* §§ 13520, 13521 (West. 1971).) CDPH's charge is to set levels of constituents "which will result in reclaimed water safe from the standpoint of public health, for the uses to be made." (*Id.* § 13520 (West. 1971).)

"'Recycled water' means water which, as a result of treatment of waste, is suitable for a direct beneficial use or a controlled use that would not otherwise occur and is therefor considered a valuable resource." (Wat. Code, § 13050, subd. (n).) CDPH adopted regulations defining different types of recycled water, depending on the level of treatment, such as disinfected tertiary

[3] On June 3, 2014, SWRCB adopted the General WDRs. On July 1, 2014, CDPH's Drinking Water Program was moved into the State Water Board. (Health & Saf. Code, § 116271; Sen. Bill No. 861 (Reg. Sess. 2013-2014) § 63.)

recycled water, disinfected secondary-2.2 recycled water, disinfected secondary-23 recycled water, and undisinfected secondary recycled water. (Cal. Code Regs., tit. 22, §§ 60301.220, 60301.225, 60301.230, 60301.900.) Water Code section 13521 provides: "The State Department of Public Health shall establish uniform statewide recycling criteria for each varying type of use of recycled water where the use involves the protection of public health."

CDPH's permitting the use of recycled water to irrigate citrus crops is nothing new. Consistent with its statutory mandate in Water Code section 13521, CDPH adopted regulations, most recently amended in 2000, which allow for the particular uses of different types of recycled water. As relevant to Petitioner's petition for writ of mandate, CDPH's regulation provides that "undisinfected secondary recycled water" is safe from a public health perspective for use to irrigate orchards. (Cal. Code Regs., tit. 22, § 60304, subd. (d).) CDPH's regulations include sampling and analysis requirements for different types of recycled water. (*Id.* § 60321.) Those regulations do not require sampling and analysis for undisinfected secondary recycled water. (*Id.*) The regulations also require that an engineering report be prepared by a qualified engineer licensed in California which clearly indicates the means for compliance with these regulations and includes a contingency plan to assure that no untreated or inadequately treated recycled water be delivered. (*Id.* § 60323.) This is commonly known as a "title 22 engineering report." Like all regulations, CDPH adopted that regulation following formal notice and comment rulemaking, and the regulation was approved by the Office of Administrative Law. OAL's approval was published in its California Regulatory Notice Register 2000, No. 44. (*Id.*) Thus, almost 15 years ago CDPH found that irrigating citrus crops with recycled water is "safe from the standpoint of public health." (Wat. Code, § 13520 (West. 1971); Cal. Code Regs., tit. 22, § 60304, subd. (d).)

///

///

///

B. The State Water Board's Regulation of Recycled Water

1. Waste Discharge Requirements

The State Water Board, and the nine regional water boards, are the "principal state agencies with primary responsibility for the coordination and control of water quality." (Wat. Code, § 13001; *California Sportfishing Protection Alliance v. State Water Resources Control Bd.* (2008) 160 Cal.App.4th 1625, 1638; RJN Ex. B [1996 MOA at p. 2].) Recycled water users are required to submit reports of waste discharge and obtain permits from the regional water boards to permit the particular uses of the recycled water produced at a particular facility. (Wat. Code, § 13522.5, 13523, 13523.1.) Discharges of recycled water without a permit are prohibited. (Wat. Code, § 13529.2.) A regional board may issue "waste discharge requirements" (i.e., a permit) to an individual discharger. (Wat. Code, § 13263, subd. (a).) In years past, individual recycled water producers would generally obtain permission to distribute recycled water by seeking and obtaining individual WDRs from their local regional board.

The Legislature also empowered a regional board, or the State Water Board, to adopt "*general* waste discharge requirements for a category of discharges" under certain circumstances. (Wat. Code, § 13263, subds. (i) and (j).) General WDRs are essentially region-wide or state-wide generic permit terms that dischargers can elect to utilize to permit their discharges by enrolling in the general permit, rather than initiating an individual WDR permitting process. Other than in the San Francisco region,[4] prior to the State Water Board's adoption of General WDRs in June 2014, recycled water users were required to apply for and obtain waste discharge requirements for their particular facility from their local regional board.

2. The Recycled Water Policy

In 2009, the State Water Board adopted the Policy for Water Quality Control for Recycled Water (Recycled Water Policy), which was amended in 2013. (AR 400-440.) The Recycled Water Policy and its amendment were adopted as a regulation after regular notice and comment

[4] The San Francisco regional board has utilized general waste discharge requirements to permit recycling facilities in its region since 1996. (<<http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/wastewaterrecyclingandreuse.shtml>>.)

period, and subsequently approved by the Office of Administrative Law. (Cal. Code Reg., tit. 23, § 2920.) The Recycled Water Policy, consistent with direction from the Legislature, establishes the goal of increasing the use of recycled water by at least 1 million acre feet per year by 2020, and by at least 2 million acre feet per year by 2030. (AR 405.) The State Water Board, consistent with prior Legislative findings, declared that it is "waste" and "unreasonable use" of water to use potable water when recycled water of adequate quality is available. (AR 408, citing Wat. Code, § 13550.) The policy further identifies criteria by which permits for recycled water use will be issued. (AR 407.) Among other criteria, the Recycled Water Policy provides that all uses of recycled water must meet health and safety requirements set by CDPH. (AR 418.)

III. CALIFORNIA'S CURRENT DROUGHT AND THE GOVERNOR'S RESPONSE

In 2014 the State was enduring its third year of extreme drought. Potable water supplies were stretched thin. Some communities had run out of potable water. Water deliveries to farmers were slashed. Drought-caused wildfires were increasing. Lower stream flows were endangering already endangered fish and wildlife species. The Sacramento-San Joaquin Delta was threated with increased salt water intrusion, imperiling freshwater Delta supplies. (AR 395.)

Against this backdrop, on January 17, 2014, Governor Brown declared a state of emergency due to severe drought conditions. (AR 5, 395; Gov. Code, § 8558, subd. (b).) The January 2014 Executive Order required a number of conservation activities to decrease water demand, and other actions to increase and reallocate water supply. (AR 5, 395.) State Water Board staff began work on general waste discharge requirements for recycled water to encourage the increased use of recycled water in order to conserve potable water supplies by streamlining the recycled water permitting process. (AR 127, 193.)

After three more months of drought, the Governor determined that more emergency actions were necessary. On April 25, 2014, in reliance on his powers pursuant to the California Emergency Services Act (Gov. Code, § 8550, et seq.), the Governor found that "expedited actions" are needed to mitigate the drought's harmful impacts. (AR 5, 396.) The Governor ordered fifteen expedited actions, including:

- speeding administrative review of water transfer requests,

- various actions to conserve water in domestic and commercial settings,
- actions to protect aquatic species,
- assisting local agencies vulnerable to acute water shortages,
- providing incentives to reduce water demands by agricultural users, and
- actions to reduce the threat of wildfire caused by drought conditions.

(AR 395-399.) Of most relevance here, the Governor also directed the State Water Board to "adopt statewide general waste discharge requirements to facilitate the use of treated wastewater [i.e., recycled water] that meets standards set by the Department of Public Health, in order to reduce demand on potable water supplies." (AR 397 ¶ 10.) Among the Governor's powers during a state of emergency is the authority to suspend any regulatory statute which "would in any way prevent, hinder, or delay the mitigation of the effects of the emergency." (Gov. Code, § 8571.) Because of the emergency need to implement the expanded use of recycled water in the state, which would reduce the strain the drought was placing on potable water sources, and "to allow these actions to take place as quickly as possible," the Governor declared that the State Water Board's action was exempt from CEQA. (AR 398 ¶ 19.)

IV. THE STATE WATER BOARD'S 2014 GENERAL WDRS

In compliance with the Governor's Executive Order, on April 29, 2014, the State Water Board released *Draft General Waste Discharge Requirements for Recycled Water Use* for public comment. (AR 89-124.) 34 members of the public submitted written comments, including Petitioner. (AR 451-885, 620-772 [Petitioner's comments].)

The General WDRs function as a permit to allow the production and distribution of recycled water for non-potable uses, such as landscape and agricultural irrigation and certain industrial uses (such as cooling towers). (AR 195.) The General WDRs do not permit direct potable uses (i.e., sending recycled water directly to retail distribution systems), or indirect potable uses (such as groundwater recharge) (AR 195); those uses still require individual WDRs. The General WDRs impose numerous prohibitions, including a prohibition on causing nuisance, limits on when irrigation may occur, and a prohibition on recycled water leaving the place of use. (AR 17-18.) The General WDRs also affirmatively impose a number of requirements, including

a requirement to comply with all CDPH regulations found in California Code of Regulations titles 17 and 22, preparation of a title 22 engineering report, preparation of a salt and nutrient management plan approved by a regional water board, and others. (AR 18-19.) Applicants seeking coverage under the General WDRs must submit a notice of intent to enroll, and must comply with a laundry list of administrative reporting, notification, education, monitoring and maintenance requirements. (AR 19-22.)

On June 3, 2014, the State Water Board conducted a public hearing on the draft General Order. (AR 125-126 [agenda]; AR 127-162 [staff report]; AR 163-390 [hearing transcript].) Petitioner did not appear at the hearing. (AR 163-390; Opening Brief at p. 3:1.) After receiving and reviewing comments made during the hearing, the State Water Board staff prepared Change Sheet 2 to revise the General WDRs in response to certain comments. (AR 39-43, 322-323.) All the comments received, including Petitioner's, were provided to the State Water Board members (AR 197), and before rending a final decision, the State Water Board acknowledged that it "heard and considered all comments." (AR 17.) At the conclusion of the public hearing, the State Water Board adopted Water Quality Order 2014-0090 General Water Discharge Requirements for Recycled Water Use (General WDRs). (AR 1-38.) On July 3, 2014, Petitioner timely filed the instant petition for writ of mandate challenging the General WDRs.

STANDARD OF REVIEW

The State Water Board adopted the General WDRs pursuant to Water Code section 13263. Actions taken pursuant to that Water Code section are reviewed by a petition for writ of mandate. (Wat. Code, § 13330, subd. (a).) Petitions for writ of mandate filed pursuant to Water Code section 13330 are reviewed pursuant to Code of Civil Procedure section 1094.5. (Wat. Code, § 13330, subd. (d).) The Court's review is limited to whether the State Water Board (1) exceeded its jurisdiction, (2) whether there was a fair trial, or (3) whether there was an prejudicial abuse of discretion. (Code Civ. Proc., § 1094.5, subd. (b).) In this case, Petitioner argues that the State Water Board abused its discretion. (Opening Brief at pp. 11-13.) Abuse of discretion is established if the court determines that the State Water Board's findings are not supported by substantial evidence in light of the whole record. (Code Civ. Proc., § 1094.5, subd. (c).) The

Court is not directed to exercise its independent judgment in its review of this agency action. (Wat. Code, § 13330, subd. (d) [independent review is limited to actions taken by a regional board or the State Water Board's review of a regional board action]; Code Civ. Proc., § 1094.5, subd. (c).)

In this case, Petitioner claims that the State Water Board abused its discretion because, according to him, the State Water Board's Order is not supported by the findings, and the findings are not supported by substantial evidence, as required by *Topanga Assn. for a Scenic Community v. County of Los Angeles* (1974) 11 Cal.3d 506, 515. (Petitioner's Opening Brief at pp. 11-13.) *Topanga* requires that the agency's findings disclose "the analytic route the administrative agency traveled from evidence to action." (*Topanga, supra*, 11 Cal.3d at p. 515.) "In making these determinations, the reviewing court must resolve reasonable doubts in favor of the administrative findings and decision." (*Id.* at p. 514.)

ARGUMENT

I. THE STATE WATER BOARD DID NOT ABUSE ITS DISCRETION BECAUSE ITS FINDINGS ARE SUPPORTED BY SUBSTANTIAL EVIDENCE IN LIGHT OF THE WHOLE RECORD

A. The State Water Board's General WDRs Are Supported By the Finding That the General WDRs Are Safe From a Human Health Perspective

The State Water Board may adopt General WDRs if it satisfies the legislative requirements set forth in Water Code sections 13263 and 13241. (Wat. Code, §§ 13263, subd. (a), 13241.) These elements include the beneficial uses to be protected, the water quality objectives reasonably required for that purpose, other waste discharges, the need to prevent nuisance, past, present, and probable future beneficial uses, environmental characteristics, water quality conditions that could reasonably be achieved through the coordinated control of all factors which affect water quality in the area, economic considerations, the need for developing housing, and the need to develop and use recycled water. (*Id.*) According to *Topanga*, the State Water Board's action of adopting the General WDRs can be sustained if the findings support the conclusion that all of these legislative requirements have been satisfied. (*Topanga, supra*, 11 Cal.3d at pp. 511, 518.) Assuming, without conceding, that Water Code section 13263(a)'s

requirement to consider "the need to prevent nuisance" encompasses a requirement to consider the General WDRs' potential human health impacts, the State Water Board satisfied that requirement.

Paragraphs 9 and 10 of the General WDRs contain an express finding that recycled water is safe from a human health perspective for approved uses.[5] In pertinent part, the State Water Board found as follows:

> 9. The CDPH has primary statewide responsibility for protecting public health. It has established statewide water recycling criteria in California Code of Regulations, title 22, division 4, chapter 3 (hereafter referred to as title 22). Approved uses of recycled water under title 22 depend on the level of treatment, disinfection, and potential for public contact. CDPH has categorized recycled water based on treatment and disinfection levels. There are four categories of recycled water relevant to this General Order; they are listed here and defined in the indicated title 22 section:
>
> a. Undisinfected secondary recycled water (Cal. Code Regs., tit. 22, § 60301.900.)
>
> b. Disinfected secondary-23 recycled water (Cal. Code Regs., tit. 22, § 60301.225.)
>
> c. Disinfected secondary-2.2 recycled water (Cal. Code Regs., tit. 22, § 60301.220.)
>
> d. Disinfected tertiary recycled water (Cal. Code Regs., tit. 22, § 60301.230.)
>
> An approved title 22 engineering report is required before authorization to use recycled water is granted by the Executive Officer.
>
> 10. When used in compliance with the Recycled Water Policy, title 22, and all applicable state and federal water quality laws, *the State Water Board finds that recycled water is safe for approved uses*, and strongly supports recycled water as a safe alternative to raw and potable water supplies for approved uses.

(AR 6-7, italics added.)

---

[5] Petitioner curiously cites to the State Water Board's finding in Paragraph 25 of the Order that "by restricting the use of recycled water to title 22 requirements, this order ensures that recycled water is used safely." (Petitioner's Opening Brief, at p. 11, citing AR 13.) It is unclear why Petitioner cites to this finding, as it is a finding that the General WDRs will not degrade water quality, not that the General WDRs will not have adverse human health impacts. (See AR 10-15, ¶¶ 22-28 [under the heading "Antidegradation Analysis"].) As such, this finding was not made in support of the conclusion that recycled water was safe from a human health perspective, but instead was made to support the conclusion that the General WDRs would not degrade water quality in violation of the State Water Board's antidegradation policy. This Respondent's Brief will focus on the findings made in Paragraphs 9 and 10 made in relation to human health impacts.

Petitioner cannot credibly argue that the State Water Board did not make a finding that adoption of the General WDRs is safe from a human health perspective. As the Supreme Court held, findings do not need to be extensive or detailed. (*Environmental Protection Information Center v. Cal. Dept. of Forestry and Fire Protection* (2008) 44 Cal.4th 459, 516-517.) "[W]here reference to the administrative record informs the parties and reviewing courts of the theory upon which an agency has arrived at its ultimate finding and decision it has long been recognized that the decision should be upheld if the agency 'in truth found those facts which as a matter of law are essential to sustain its ... [decision].'" (*Sierra Club v. California Coastal Commission* (1993) 19 Cal.App.4th 547, 556.) The State Water Board's findings are sufficient.

Petitioner's argument instead appears to be that the State Water Board was required to make additional, more narrow and specific findings with respect to the potential human health impacts of the potential for perchlorate to be present in recycled water used to irrigate citrus crops in response to Petitioner's comment letter. (Opening Brief at pp. 11-12.) There is no requirement that the State Water Board was required to adopt such narrow, specific finding as to one particular chemical identified in Petitioner's comment letter. Following Petitioner's logic, the State Water Board would be required to make specific findings with respect to every single issue raised in every single comment letter it received, turning the findings (which are supposed to be tied to the legislative requirements set forth in Water Code section 13263) into an unwieldy, detailed response to comments. The State Water Board received 34 separate written comments letter, which totaled 434 pages (AR 451-885), and conducted a multi-hour public hearing (AR 163-390). The vast majority of the comments supported the State Water Board's adoption of General WDRs, but many suggested revisions were made. (*Id.*) Taking every individual comment and making a finding as to why the State Water Board did not change its order in response to that comment would turn the process of adopting findings that support the decision into an unwieldy requirement that does not presently exist in the law.

For most projects that trigger CEQA, the agency is required to prepare written responses to comments. (Pub. Resources Code, § 21091, subd. (d)(2).) That requirement is absent here because the Governor exempted this project from CEQA pursuant to his emergency powers. (AR

5, 396.) The requirement to prepare written responses to comments is a requirement unique to CEQA, and is not otherwise required by the Code of Civil Procedure section 1094.5 or *Topanga*. While the State Water Board is required to explain the analytical route it traveled, as *Topanga* requires, it is not required to adopt findings to explain why it did *not* travel the route that Petitioner suggested in this comments.

B. The State Water Board's Findings Are Supported by Substantial Evidence

The State Water Board's finding that the use of recycled water in conformance with existing laws and regulations is safe from a human health perspective is supported by substantial evidence. (Code Civ. Proc., § 1094.5, subd. (c); Wat. Code, § 13330, subd. (d) [substantial evidence, not independent review, is applicable standard of review].) The Court is to presume that the State Water Board's findings are supported by substantial evidence. (*Desmond v. County of Contra Costa* (1993) 21 Cal.App.4th 330, 335.) "All presumptions are indulged and conflicts resolved in favor of the Board's decision." (*Furtado v. State Personnel Board* (2013) 212 Cal.App.4th 729, 742.) The Petitioner has the burden to show that there is no substantial evidence to support the State Water Board's decision. (*Young v. Gannon* (2002) 97 Cal.App.4th 209, 225.) "When more than one inference can be reasonably deduced from the facts, we cannot substitute our own deductions for that of the agency. [citation] We may reverse an agency's decision only if, based on the evidence before it, a reasonable person could not have reached such decision." (*Donley v. Davi* (2009) 180 Cal.App.4th 447, 456.)

The State Water Board's finding regarding potential human health impacts of using recycled water for specified applications is based on CDPH's regulations regarding same. CDPH's regulations constitute substantial evidence to support the State Water Board's findings. As stated in the administrative record, CDPH has "primary statewide responsibility for protecting public health." (AR 6.) Indeed, the State Water Board had consistently relied "on the expertise of CDPH for the establishment of permit conditions needed to protect human health." (AR 409.) The State Water Board recognized that CDPH has already made determinations as to the safe uses of different types of recycled water for different applications, including use of undisinfected secondary recycled water to irrigate orchards. (Cal. Code Regs., tit. 22, § 60301.900.)

It is wholly appropriate for one agency to rely on the expertise of another agency when making decisions that implicate that other agency's area of expertise. Another agency's regulations can provide substantial evidence in support of an agency's findings. For example, in *Oakland Heritage Alliance*, a petitioner claimed that the City of Oakland's finding that a development project was safe from a geotechnical standpoint was not supported by substantial evidence. (*Oakland Heritage Alliance v. City of Oakland* (2011) 195 Cal.App.4th 884, 903-904.) The Court disagreed, holding that the City's reliance on the Building Code's seismic provisions (a regulation) was substantial evidence to support the City's finding that compliance with the Building Code would reduce the earthquake risks to a less than significant level. (*Id.*; see also *Tracy First v. City of Tracy* (2009) 177 Cal.App.4th 912, 930-931 [agency's requirement to comply with state energy efficiency standards was substantial evidence in support of finding that project would not have significant energy impact].)

Further, the State Water Board's finding are made against the backdrop of the Governor's Executive Order, which expressly directed the State Water Board to adopt General WDRs "that meet standards set by the Department of Public Health." (AR 397 ¶10.) The Governor expressly directed the State Water Board to rely on the human health standards established by CDPH. Petitioner in essence is asking the State Water Board to ignore the Governor's direction, and to override CDPH's determination that undisinfected secondary recycled water is safe for use in orchards. Such a conclusion would not be consistent with adopting General WDRs that meet "standards set by the Department of Public Health." (*Id.*)

II. PETITIONER HAS ANOTHER FORUM IN WHICH HE CAN SEEK RELIEF

Petitioner's petition is nothing more than a disguised collateral attack on CDPH's regulation in which CDPH previously concluded that it is safe from a human health perspective to irrigate citrus orchards with undisinfected secondary recycled water. (Cal. Code Regs., tit. 22, § 60304.) Determining whether the use of undisinfected secondary recycled water to irrigate citrus orchards is a safe from a human-health perspective is a scientific determination vested within CDPH's jurisdiction. (Wat. Code, § 13521.)

If Petitioner believes regulations which allow the use of undisinfected secondary recycled water in orchards without monitoring for perchlorate should be revisited, Petitioner has other administrative remedies that he can pursue. For example, Petitioner "may petition a state agency requesting the adoption, amendment, or repeal of a regulation." (Gov. Code, § 11340.6.) A state agency receiving such a petition is required to respond within 30 days as to whether to grant or deny the petition, and may take any other action as it deems warranted by the petition. (*Id.* § 11340.7.) What Petitioner cannot do is obtain judicial review of CDPH's regulation through a challenge to the State Water Board's General WDRs that relies on that regulation.

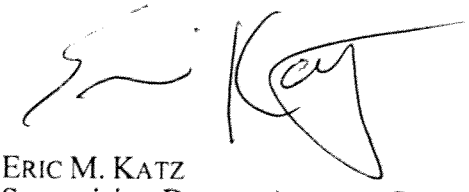
CONCLUSION

The State Water Board adopted General WDRs to streamline the permitting of recycled water facilities as an emergency drought response measure in compliance with the Governor's April 25, 2014 executive order. CDPH years ago determined that it was safe from a human health perspective to irrigate citrus crops with recycled water. In reliance on that regulatory determination made by the state agency specifically vested with jurisdiction to make it, the State Water Board found that the use of recycled water in compliance with CDPH's regulation is safe. The State Water Board's finding is supported by substantial evidence and therefore should not be disturbed. Although Petitioner believes that there is evidence that would support a different conclusion, a difference of opinion does not mean that the State Water Board's finding is not supported by substantial evidence. The State Water Board respectfully requests that the petition for writ of mandate be denied.

Dated: May 28, 2015

Respectfully Submitted,

KAMALA D. HARRIS
Attorney General of California



ERIC M. KATZ
Supervising Deputy Attorney General
Attorneys for Respondent
State Water Resources Control Board

LA2014512195
51754678_2.doc

DECLARATION OF SERVICE BY U.S. MAIL

Case Name: **Andrew C. Wilson v. State Water Resources Control Board**

Case No.: **BS149632**

I declare:

I am employed in the Office of the Attorney General, which is the office of a member of the California State Bar, at which member's direction this service is made. I am 18 years of age or older and not a party to this matter; my business address is 300 South Spring Street, Suite 1702, Los Angeles, CA 90013.

On May 28, 2015, I served the attached **RESPONDENT'S OPPOSITION BRIEF** by placing a true copy thereof enclosed in a sealed envelope with postage thereon fully prepaid, in the United States Mail at Los Angeles, California, addressed as follows:

Mr. Andrew C. Wilson
7468 Dufferin Ave.
Riverside, California 92504
In Pro Se

I declare under penalty of perjury under the laws of the State of California the foregoing is true and correct and that this declaration was executed on May 28, 2015, at Los Angeles, California.

Beatriz Davalos
Declarant

Beatriz Davalos
Signature

LA2014512195
51680257.doc

EXHIBIT 5

ANDREW C. WILSON
State Bar No. 133062
7468 Dufferin Avenue
Riverside, CA 92504
Telephone: (951) 687-4471
E-mail: acwilson11@yahoo.com

Petitioner In Pro Se

SUPERIOR COURT OF THE STATE OF CALIFORNIA

COUNTY OF LOS ANGELES

CENTRAL DISTRICT

ANDREW C. WILSON, Petitioner. **v.** **STATE WATER RESOURCES CONTROL BOARD,** Respondent.	Case No. BS149632 **PETITIONER'S REPLY BRIEF** Dept: 85 Judge: The Honorable James C. Chalfant Trial Date: July 28, 2015 1:30 p.m. Action Filed: July 3, 2014

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Cases

Statutes

Regulations

I. INTRODUCTION

One of the key purposes of this adjudicatory process, the proposed general permit, and the comments and hearing on it, was to determine if recycled water was safe enough for broad use under a statewide general permit, as opposed to individual permits. Despite this, the State Board is claiming that no matter what scientists have discovered about recycled water contaminants and their damaging effects, specifically the effects of perchlorate, a conclusion the California Department of Public Health (CDPH) reached about recycled water 15 years ago allowed the State Board to ignore such scientific evidence. The State Board's position makes a mere formality of the very process on which it embarked.

II. ARGUMENT

A. Water Quality Order 2014-0090 (The Order) Is Not Supported By The Findings.

The Order is not supported by the findings as required by *Topanga Assn. for a Scenic Community v. County of Los Angeles* (1974) 11 Cal.3d 506, 518 [113 Cal.Rptr. 836] because the State Board's decision to reject Petitioner's argument does not logically follow from the findings.

When an agency's decision does not follow as a matter of law from the findings, the decision must be set aside. The Supreme Court in *Topanga* cited the case *Saginaw Broadcasting Co. v. Federal C. Com'n* (1938) 96 F.2d 554. (*Topanga*, 11 Cal.3d at p. 516.) The *Saginaw* court explained: "When a decision is accompanied by findings of fact, the reviewing court can decide whether the decision reached by the court or commission follows as a matter of law from the facts stated as its basis." (*Saginaw*, *supra*, 96 F.2d at p. 559, italics added.)

According to the State Board, the following finding sets forth its basis for rejecting Petitioner's argument about perchlorate: "When used in compliance with the Recycled Water Policy, title 22, and all applicable state and federal water quality laws, the State Water Board finds that recycled water is safe for approved uses, and strongly supports recycled water as a safe alternative to raw and potable water supplies for approved uses." (AR 7.)

As interpreted by the State Board in its Opposition Brief, the finding discloses the following mode of analysis that the State Board used in rejecting Petitioner's argument. Fifteen years ago the California Department of Public Health (CDPH) adopted title 22 § 60304, which

imposes treatment standards for recycled water used for crop irrigation. Based on that fact, the State Board inferred that 15 years ago the CDPH also determined that irrigating crops with recycled water that has been treated in compliance with title 22 § 60304 is safe for human health, including safe against the risk of perchlorate.

However, it is not logical or reasonable to infer that title 22 protects against perchlorate when the regulation never mentions perchlorate. The treatment standards pertinent to title 22 § 60304 specifically address other constituents in the water, including "turbidity" as well as harmful microbes (such as bacteria and viruses). The law requires inferences to be both logical and reasonable. (See, e.g., Evidence Code § 600(b).) A reasonable inference cannot be drawn, based on the text of title 22 § 60304, that the treatment standards will reduce the level of perchlorate in the water.

It appears that the State Board on appeal concedes that the highest treatment standard imposed by title 22 § 60304 for crop irrigation, the "disinfected tertiary recycled water" standard, does not reduce perchlorate levels in the water. In its Opposition Brief the State Board never states that the treatment standards of title 22 § 60304 protect against perchlorate.

Moreover, Petitioner based his argument on facts and data drawn from four scientific articles that were published many years after the CDPH adopted title 22, § 60304 in the year 2000. Two of Petitioner's articles were published in 2006, one in 2008, and one in 2012. (AR 625, 633, 688, and 695.) It is not logical to infer from the CDPH's determination that Petitioner's

---

[1] Title 22, § 60304 provides in pertinent part:

"(a) Recycled water used for the surface irrigation of the following shall be a disinfected tertiary recycled water . . . :

"(1) Food crops, including all edible root crops, where the recycled water comes into contact with the edible portion of the crop.

* * *

"(d) Recycled wastewater used for the surface irrigation of the following shall be at least undisinfected secondary recycled water:

"(1) Orchards where the recycled water does not come in contact with the edible portion of the crop."

Treatment standards are set out in the definitions associated with "disinfected tertiary recycled water" and "undisinfected secondary recycled water." (Title 22, §§ 60301.230, 60301.900.)

argument lacks merit when the CDPH did not consider those scientific articles and data when it formulated its determination 15 years ago.

The State Board has never stated that title 22, § 60304 constituted conclusive evidence on the issue of public health. Instead it argues: "The State Board *reasonably relied* on its sister agency's human health determination," indicating that the State Board exercised its discretion in deciding whether to rely on the CDPH's determination when resolving the issue of public health. (Respondent's Brief, pp. 1-2, italics added.)

B. <u>The Findings Are Not Supported By Substantial Evidence</u>.

The only evidence the State Board relied on was the CDPH regulation (title 22 § 60304), and that regulation does not constitute substantial evidence for the findings. The State Board argues that compliance with regulations may constitute substantial evidence and cites two cases: *Oakland Heritage Alliance v. City of Oakland* (2011) 195 Cal.App.4th 884, 903-904 [124 Cal.Rptr.3d 755] ("*Oakland Heritage*") and *Tracy First v. City of Tracy* (2009) 177 Cal.App.4th 912, 930-931 [99 Cal.Rptr.3d 621] ("*Tracy First*"). Both cases are distinguishable on their facts and do not support the State Board's position. In both cases, the provisions of the safety standard in question specifically addressed and protected against the risk identified by the petitioners.

In *Oakland Heritage* the petitioner, who contested the approval of a development project, argued that there was insufficient evidence to support the city's finding that mitigation measures reduced seismic risks to less than a significant level. The petitioner argued that the mitigation measures only addressed seismic risk to human life and ignored risk to structures. The Court stated: "We do not read the Revised EIR as ignoring impacts to structures." (*Oakland Heritage Alliance*, 195 Cal.App.4th at p. 898.) The mitigation measures required, among other things, compliance with the California Building Code. The Court concluded, based on the provisions of the Building Code, that the Code protected against not only seismic risk to life, but also risk to structures: "[T]he relevant provisions of the Building Code are intended to promote structural safety in the event of an earthquake." (*Id.*, at p. 904.) The Court held that compliance with the Building Code (together with the other mitigation measures) constituted substantial evidence that the mitigation measures would reduce the seismic risk to structures to less than a significant level.

Unlike the regulations in this case, which make no mention of perchlorate, in *Oakland Heritage* the text of the Building Code addressed the specific risk that concerned the petitioner in that case, namely, the seismic risk to structures.

In *Tracy First* the petitioner contested a rezoning of property allowing 95,900-square-foot grocery store, arguing that it was improper for the City of Tracy to rely on compliance with state building standards to find that the project's "energy impact" was not significant. The City had found that the energy impact of the project was not significant because the project would meet and exceed the California Energy Efficiency Standards set forth in title 24 of the California Code of Regulations. The Court agreed that the California Energy Efficiency Standards protected against energy impacts. "The California Building Energy Efficiency Standards are meant to promote energy efficiency, as the name implies. In other words, they 'reduce the wasteful, inefficient, and unnecessary consumption of energy.'" (*Tracy First*, 177 Cal.App.4th at pp. 933-934.)

In *Tracy First*, as in *Oakland Heritage*, the provisions of the building standard at issue protected against the risk that the petitioners identified. Therefore, compliance with the standard was evidence that the risk would be abated. It follows that if the provisions of the code or building standard did not protect against the risk, then compliance with the standard would not be relevant evidence that the risk would be abated.

In the instant case, the provisions of title 22 § 60304 do not protect against perchlorate. The treatment standards do not mention perchlorate, or include limits for perchlorate, and a reasonable inference cannot be drawn that the required treatments for turbidity and microbial constituents will reduce perchlorate. Neither the State Board staff nor anyone else introduced any evidence showing that title 22 § 60304 protects against the risk of perchlorate.

Moreover, the substantial evidence standard requires the reviewing court to consider all relevant evidence in the record, including evidence that fairly detracts from the evidence supporting the agency's decision. Petitioner introduced evidence that the highest treatment standard of title 22 § 60304 will not remove perchlorate. Under the regulations, the treatment process for "disinfected tertiary treated water" involves settling solids, oxidation, filtration to

reduce turbidity, and disinfection. As explained in Petitioner's Opening Brief, the evidence shows that perchlorate exists in water is a dissolved anion, and none of these processes reduce perchlorate.

None of the factual assertions about perchlorate and the treatment processes made in Petitioner's Opening Brief were disputed by the State Board in its Opposition Brief. The failure to contest the assertions indicates a willingness to concede the points. "A party's failure to deny a statement of fact in his adversary's brief may result in the court's acceptance of that fact as true if the record does not show otherwise." (*Midwife v. Bernal* (1988) 203 Cal.App.3d 57, 63 [249 Cal.Rptr. 708]; *Garamendi v. Executive Life Ins. Co.* (1993) 17 Cal.App.4th 504, 509 n.6 [21 Cal.Rptr.2d 578].) (It is noteworthy that the instant case is about perchlorate, and yet the word "perchlorate" appears only twice in the 14 page Respondent's Opposition Brief. The first time it appears is at page 11, and the second time is at page 14.)

C. <u>None Of The State Board's Remaining Arguments Support Its Decision</u>.

(1) Findings Are Required When, As In This Case, A Party Introduces Evidence That Supports A Finding In The Party's Favor.

The State Board defends its complete failure to address perchlorate by arguing that: "Following Petitioner's logic, the State Board would be required to make specific findings with respect to every single issue raised in every single comment letter it received . . . ." (Respondent's Opposition Brief, p. 11.)

The Petitioner does not argue that there must be findings on every issue raised in every comment letter. The adequacy of findings is evaluated with "'reference to the administrative record.'" (*Environmental Protection Information Center v. Cal. Dept. of Forestry and Fire Protection* (2008) 44 Cal.4th 459, 516 [80 Cal. Rptr.3d 28; 187 P.3d 888], quoting *Sierra Club v. California Coastal Com.* (1993) 19 Cal.App.4th 547, 556 [23 Cal.Rptr.2d 534].) Those who submit comments are entitled "to present their evidence and arguments." (*Topanga, supra*, 11 Cal.3d at p. 518.) There is no need for an agency to make a finding if a comment letter presents no supporting evidence -- in those situations reference to the record shows that no finding is necessary. But, if the argument is supported by evidence, as in this case, findings must be made

that support the decision rejecting the argument to allow the party to determine whether and on what basis to appeal.

> "[I]t is a respected general rule that when there is no evidence in the record which would have supported a finding favorable to an appellant, the failure to make a finding on the subject does not constitute error. As is said in 2 Witkin, California Procedure, Trial, section 119, page 1851: 'The appellant may justly complain if he introduces evidence sufficient to support a finding in his favor on a material issue, and the court fails to make any finding thereon. But if he produces no evidence on the issue, or the evidence is such that the finding would necessarily be adverse to him, he suffers no prejudice from the failure to make it, and there is no reversible error. [Citing cases.]'" (*J.C. Wattenbarger & Sons v. Sanders* (1963) 216 Cal.App.2d 495, 504 [30 Cal.Rptr. 910] (citations omitted); *Kazensky v. City of Merced* (1998) 65 Cal.App.4th 44, 67-68 [76 Cal.Rptr.2d 356].)

In this case Petitioner submitted evidence that supported his argument. Petitioner's evidence showed the harmful effects of perchlorate. In addition to the fact that the provisions of title 22 § 60304 do not mention perchlorate, Petitioner's evidence showed that the disinfected tertiary treatment standard does not remove perchlorate. The State Board was therefore required to make findings supporting its decision rejecting Petitioner's argument.

(2) The Governor's Executive Order Did Not Prevent The State Board From Deciding This Case Based On The Evidence.

The State Board argues that Petitioner is asking it to ignore the Governor's Executive Order, which directed the State Board to adopt waste discharge requirements "that meet the standards set by the Department of Public Health." (Respondent's Brief, p. 13.) The Executive Order is properly construed to impose a minimum requirement, and not to prevent the State Board from deciding that additional safety measures may be needed when evidence at the hearing shows that issuing the general permit will threaten the public health. Also, nothing prevented the State Board from excluding oranges from the scope of the general permit.

(3) The 1996 Memorandum of Agreement Does Not Authorize The State Board To Draw Unreasonable Inferences From Title 22 § 60304.

The State Board argues that the CDPH is the "primary" state agency responsible for protection of public health, and therefore the State Board reasonably relied on the CDPH's prior determination. This argument is based on the following statement: "The [CDPH] is the primary State agency responsible for protection of public health and the regulation of drinking water,"

which appears in a 1996 "Memorandum of Agreement" (MOA) signed by the Director of the CDPH and the Executive Director of the State Board. (MOA at p. 2.)[2]

Regardless of which agency is the "primary" agency, that statement in the MOA should not be interpreted to grant the State Board legal authority to draw unreasonable inferences from the text of title 22 § 60304. Such a construction would render the MOA unlawful and void. It is unclear how the statement in the MOA is relevant to the question of whether the State Board's inferences based on the text of title 22 § 60304 are logical.

The State Board is responsible for determining whether a risk to public health will result from using recycled water for the purpose of providing water to animals, including cows, sheep, goats and pigs, and if so, it is responsible for developing through rulemaking uniform statewide recycling criteria for that use. (Water Code § 13521.1(f).) The Legislature has also moved CDPH's Drinking Water Program to the State Board. (Resonent's Brief, p. 3 n.3.) An actual or claimed lack of institutional expertise does not create authority to draw illogical inferences from the text of title 22 § 60304.

(4) The Legislature Conferred On The State Board The Responsibility To Decide The Issue Of The Permit's Effect On Human Health.

The State Board states: "Assuming, *without conceding*, that Water Code section 13263(a)'s requirement to consider 'the need to prevent nuisance' encompasses a requirement to consider the General WDR's potential human health impacts, the State Water Board satisfied that requirement." (Respondent's Brief, pp. 9-10, italics added.) The State Board suggests that the effect of the permit's provisions on human health was not a material issue at the hearing.

The Legislature conferred on the State Board the responsibility to decide the issue of the permit's effect on human health. Human health is a material issue under Water Code section

---

[2] The purpose of the inter-agency agreement is "to eliminate overlap of activities, duplication of effort, gaps in regulation, and inconsistency of action." (MOA, at p.1.) To this end, the agreement provides a dispute and conflict resolution procedure, where conflicts between the staffs of the two agencies will be taken first to the Executive Director of the State Board, who agrees, if necessary, to meet and confer with the Chief of CDPH's Division of Drinking Water and Environmental Enforcement. (Id., at p. 8.)

13263(a). That section provides that waste discharge permits "shall take into consideration" the "need to prevent nuisance." The term "nuisance" is broadly defined:

> "'Nuisance' means *anything* which meets all of the following requirements:
> "(1) Is *injurious to health*, or is indecent or offensive to the senses, or an obstruction to the free use of property, so as to interfere with the comfortable enjoyment of life or property.
> "(2) Affects at the same time an entire community or neighborhood, or any considerable number of persons, although the extent of the annoyance or damage inflicted upon individuals may be unequal.
> "(3) Occurs during, or as a result of, the treatment or disposal of wastes." (Water Code § 13050(m), italics added.)

The phrase "injurious to health" must be construed to mean human health. Farmers using recycled water for irrigation that produce contaminated crops that are then sold would fit this definition. The crops are injurious to human health, the sales would affect a considerable number of persons, and the use of recycled water only occurs as the result of the treatment of wastes (title 22 requires treatment prior to use). The definition would apply, especially in light of "the general rule that civil statutes for the protection of the public are, generally, broadly construed in favor of that protective purpose." (*People ex rel. Lundgren v. Superior Court* (1996) 14 Cal.4th 294, 313.)

Also, neither the findings nor the hearing transcript indicate that the State Board at the hearing believed that the effect of the permit's provisions on human health was immaterial. The State Board argues that it made a finding on "human health." (Respondent's Brief, pp. 1-2.) Presumably the State Board would not have made a finding on human health unless it believed that the permit's effect on human health was an issue material to the issuance of the permit.

It is improper to defend the unsupported decision on perchlorate by suggesting that human health was not a material issue. A reviewing court, when dealing with discretionary agency action, must judge the propriety of such action solely by the grounds invoked by the agency. (*Burlington Truck Lines v. United States* (1962) 371 U.S. 156, 168-69 [9 L.Ed.2d 207, 83 S.Ct. 239] ("[C]ourts may not accept appellate counsel's post hoc rationalizations for agency action.").)

(5) "Disinfected Tertiary Recycled Water" Treatment Does Not Protect Against Perchlorate.

The State Board mistates Petitioner's argument as being limited to whether undisinfected "secondary" recycled water protects against perchlorate. (See, eg., Respondent's Brief, pp. 4, 13,

14.) Petitioner, however, asserts that disinfected tertiary recycled water fails to protect against perchlorate.

As explained in Petitioner's Opening Brief, primary treatment involves allowing solids to settle to the bottom. Secondary treatment involves bubbling oxygen through the water. (The treated wastewater in Massachusetts with perchlorate levels of 250 ppb to 700 ppb was secondary treated water. (AR 678)) Disinfected tertiary recycled water treatment involves the additional steps of filtration to reduce turbidity to specific standards, and then disinfection. None of these treatments remove perchlorate.

Petitioner is concerned that the State Board is attempting to recast Petitioner's argument as being limited to secondary treated water in an effort to protect the Board's decision that irrigating with disinfected tertiary recycled water is safe. In this proceeding, however, Petitioner challenges the Board's decision that disinfected tertiary recycled water is safe for irrigating oranges. A reasonable person would not use disinfected tertiary recycled water to irrigate oranges if the water has not been tested for perchlorate. A reasonable person would recognize the need for further investigation.

D. <u>Petitioner Has Pursued The Proper Administrative Remedy In the Proper Forum</u>.

The State Board argues that Petitioner has brought a disguised "collateral attack" against the determination the CDPH made in its rulemaking proceeding 15 years ago. The phrase "collateral attack" is traditionally associated with res judicata, and generally refers to an attempt to avoid the res judicata effect of a prior judgment in another proceeding. (*Wouldridge v. Burns* (1968) 265 Cal.App.2d 82, 84 [71 Cal.Rptr. 394].) The doctrine of res judicata "does not apply when the decision of the agency is made pursuant to its rule-making powers." (*Hollywood Circle v. Dept. of Alcoholic Beverage Control* (1961) 55 Cal.2d 728, 732 [13 Cal.Rptr. 104, 361 P.2d 712].) In any event, an argument that an agency has drawn an unreasonable inference from a regulation is not an attack on the regulation.

The State Board also argues that Petitioner has "other administrative remedies he can pursue," such as petitioning the CDPH to request the adoption, amendment, or repeal of a regulation pursuant to Government Code section 11340.6. However, that "remedy" is not a plain,

speedy, and adequate remedy within the meaning of Code of Civil Procedure section 1086, which provides: "The writ must be issued in all cases where there is not a plain, speedy, and adequate remedy, in the ordinary course of law." The issuance of the Order will create an unreasonable risk to the public health statewide. Petitioner has no other remedy to prevent the issuance of the Order. A right to petition the CDPH (or even the Legislature) does not excuse the State Board from properly performing its duties in this case, including its duty to consider all the relevant evidence fully and fairly, and to render findings of fact sufficient to support its decision. Nothing requires Petitioner to pursue or exhaust any other administrative remedies.

E. <u>The State Board's Request For Judicial Notice Should Be Denied</u>.

The State Board filed a Request for Judicial Notice of documents concurrently with its Opposition Brief. The request should be denied for lack of relevancy. The State Board does not explain how any of those documents are relevant to the questions of law before this Court.

III. CONCLUSION

For all the foregoing reasons Petitioner respectfully requests that the Order be set aside.

Respectfully submitted,

Dated: July 1, 2015

ANDREW C. WILSON

Andrew C. Wilson

Andrew C. Wilson
Petitioner In pro se

ATTORNEY OR PARTY WITHOUT ATTORNEY (Name, State Bar number, and address):
ANDREW C. WILSON
STATE BAR NO. 133062
7468 Dufferin Avenue
Riverside, CA 92504

TELEPHONE NO: (951) 687-4471 FAX NO (Optional):
E-MAIL ADDRESS (Optional): acwilson11@yahoo.com
ATTORNEY FOR (Name): In pro se

FOR COURT USE ONLY

SUPERIOR COURT OF CALIFORNIA, COUNTY OF Los Angeles
STREET ADDRESS: 111 North Hill Street
MAILING ADDRESS:
CITY AND ZIP CODE: Los Angeles, CA 90012
BRANCH NAME: Central District - Stanley Mosk Courthouse

PETITIONER/PLAINTIFF: ANDREW C. WILSON

RESPONDENT/DEFENDANT: STATE WATER RESOURCES CONTROL BOARD

PROOF OF SERVICE BY FIRST-CLASS MAIL—CIVIL

CASE NUMBER: BS149632

(Do not use this Proof of Service to show service of a Summons and Complaint.)

1. I am over 18 years of age and **not a party to this action.** I am a resident of or employed in the county where the mailing took place.

2. My residence or business address is:
   7468 Dufferin Avenue
   Riverside, CA 92504

3. On *(date)*: July 1, 2015 I mailed from *(city and state)*: Riverside, California
   the following **documents** *(specify)*:
   Petitioner's Reply Brief

   ☐ The documents are listed in the *Attachment to Proof of Service by First-Class Mail—Civil (Documents Served)* (form POS-030(D)).

4. I served the documents by enclosing them in an envelope and *(check one)*:
   a. ☑ **depositing** the sealed envelope with the United States Postal Service with the postage fully prepaid.
   b. ☐ **placing** the envelope for collection and mailing following our ordinary business practices. I am readily familiar with this business's practice for collecting and processing correspondence for mailing. On the same day that correspondence is placed for collection and mailing, it is deposited in the ordinary course of business with the United States Postal Service in a sealed envelope with postage fully prepaid.

5. The envelope was addressed and mailed as follows:
   a. **Name** of person served: Eric M. Katz, Supervising Deputy Attorney General
   b. **Address** of person served:
      300 South Spring Street, Suite 1702
      Los Angeles, CA 90013

   ☐ The name and address of each person to whom I mailed the documents is listed in the *Attachment to Proof of Service by First-Class Mail—Civil (Persons Served)* (POS-030(P)).

I declare under penalty of perjury under the laws of the State of California that the foregoing is true and correct.

Date: July 1, 2015

Margaret C. Wilson
(TYPE OR PRINT NAME OF PERSON COMPLETING THIS FORM)

Margaret C. Wilson
(SIGNATURE OF PERSON COMPLETING THIS FORM)

EXHIBIT 6

#3T

Andrew C. Wilson v. State Water Resources Control Board
BS 149632

Tentative decision on petition for writ of mandate: denied

Petitioner Andrew C. Wilson ("Wilson") seeks a writ of administrative mandate ordering Respondent State Water Resources Control Board ("Board") to set aside the order adopted on June 3, 2014, entitled "General Waste Discharge Requirements for Recycled Water Use."

The court has read and considered the moving papers, opposition, and reply, and renders the following tentative decision.

A. Statement of the Case

Petitioner Wilson commenced this proceeding on July 3, 2014. The Petition alleges in pertinent part as follows. On June 3, 2014, the Board held a public meeting to receive evidence on the issue of whether grounds exist for prescribing general waste requirements for recycled water use. Wilson submitted written comments in opposition, which were received by the Board. These written comments discussed the danger of the chemical perchlorate, and included four scientific articles as exhibits.

The Board adopted a written order on June 3, 2104, entitled "General Waste Discharge Requirements for Recycled Water Use" (the "Order"). The Board decided in the Order that compliance with the California Department of Public Health recycling criteria, set forth in CCR title 22, is sufficient to protect against public health risks arising from the use of recycled water to irrigate food crops. The Order did not discuss perchlorates, or address Wilson's comments. The Order does not contain any findings to support this conclusion.

B. Standard of Review

CCP section 1094.5 is the administrative mandamus provision which structures the procedure for judicial review of adjudicatory decisions rendered by administrative agencies. Topanga Ass'n for a Scenic Community v. County of Los Angeles, ("Topanga") (1974) 11 Cal.3d 506, 514-15.

Section 1094.5 does not in its face specify which cases are subject to independent review, leaving that issue to the courts. Fukuda v. City of Angels, (1999)20 Cal.4th 805, 811. In cases reviewing decisions which affect a vested, fundamental right the trial court exercises independent judgment on the evidence. Bixby v. Pierno, (1971) 4 Cal.3d 130, 143. See CCP §1094.5(c). In other cases, the substantial evidence test applies. Mann v. Department of Motor Vehicles, (1999) 76 Cal.App.4th 312, 320; Clerici v. Department of Motor Vehicles, (1990) 224 Cal.App.3d 1016, 1023. CCP section 1094.5 governs proceedings challenging the Board's decisions. Water Code §13330(e). The court exercises independent judgment when reviewing a Board decision from an appeal from a regional board. Id. Otherwise, the substantial evidence standard applies. Id.

"Substantial evidence" is relevant evidence that a reasonable mind might accept as adequate to support a conclusion [California Youth Authority v. State Personnel Board, (2002) 104 Cal.App.4th 575, 585] or evidence of ponderable legal significance, which is reasonable in nature, credible and of solid value. Mohilef v. Janovici, (1996) 51 Cal.App.4th 267, 305, n.28. The petitioner has the burden of demonstrating that the agency's findings are not supported by

substantial evidence in light of the whole record. Young v. Gannon, (2002) 97 Cal.App.4th 209, 225. The trial court considers all evidence in the administrative record, including evidence that detracts from evidence supporting the agency's decision. California Youth Authority, *supra*, 104 Cal.App.4th at 585.

The agency's decision must be based on the evidence presented at the hearing. Board of Medical Quality Assurance v. Superior Court, (1977) 73 Cal.App.3d 860, 862. The hearing officer is only required to issue findings that give enough explanation so that parties may determine whether, and upon what basis, to review the decision. Topanga, *supra*, 11 Cal.3d at 514-15. Implicit in section 1094.5 is a requirement that the agency set forth findings to bridge the analytic gap between the raw evidence and ultimate decision or order. Topanga, 11 Cal.3d at 515.

An agency is presumed to have regularly performed its official duties (Ev. Code §664), and the petitioner therefore has the burden of proof. Steele v. Los Angeles County Civil Service Commission, (1958) 166 Cal.App.2d 129, 137. "[T]he burden of proof falls upon the party attacking the administrative decision to demonstrate wherein the proceedings were unfair, in excess of jurisdiction or showed prejudicial abuse of discretion. Afford v. Pierno, (1972) 27 Cal.App.3d 682, 691.

C. Applicable Law

In 1969, the Legislature adopted the Water Recycling Law, declaring that the people of the state have a primary interest in the development of recycled water facilities to supplement existing water supplies. Water Code §§ 13500, 13501. The Legislature further found that the use of recycled water for agricultural uses will contribute to the "peace, health, safety and welfare" of the people of the State. Water Code §13511. Recycled water is defined as "water which, as a result of treatment of waste, is suitable for a direct beneficial use or a controlled use that would not otherwise occur and is therefore considered a valuable resource." Water Code §13050(n). The Legislature has declared that it is a waste to use potable water when recycled water of adequate quality is available. Water Code §13550. The Governor's 2014 California Water Action Plan specifically calls for the increased use of recycled water as a key step to meeting the State's water policy goals. Opp. RJN, Ex., p.7

Two state agencies have responsibilities for regulating recycled water. The Board and the nine regional water quality control boards have primary responsibility for regulating the quality of recycled water and sustaining water supplies. Water Code §13001; AR 408. Non-party California Department of Public Health ("CDPH"), formerly the Department of Health Services, has primary responsibility for setting standards to protect public health in the use of recycled water. Water Code §§ 13520, 13521. In 1996, the two agencies executed a Memorandum of Understanding delineating their respective obligations. Opp. RJN Ex. B.

1. The Board

The Porter-Cologne Water Quality Control Act (the "Act") protects the quality of the waters of the State, including both surface water and groundwater, through a permitting process that controls the discharge of "waste." The term "waste" includes sewage and all other waste substances associated with human habitation or from any producing, manufacturing, or processing operation. Water Code §13050(d). The Act requires persons to report to their local Regional

Board proposed discharges of waste that could adversely affect the quality of the State's water. Water Code §13260.

The Board and nine regional water boards are the "principal state agencies with primary responsibility for the coordination and control of water quality." Water Code §13001; California Sportfishing Protection Alliance v. State Water Resources Control Bd., (2008) 160 Cal.App.4th 1625, 1638.

In 2009, the Board, consistent with the Act, declared that it is "waste" and "unreasonable use" of water to use potable water when recycled water of adequate quality is available. *See* Water Code §13550. The policy further identifies criteria by which permits for recycled water use will be issued. AR 407. Among other criteria, the Recycled Water Policy provides that all uses of recycled water must meet health and safety requirements set by CDPH. AR 418.

Recycled water users are required to submit reports of waste discharge and obtain permits from the regional water boards to permit the particular uses of the recycled water produced at a particular facility. Water Code §§ 13522.5, 13523, 13523.1. "Recycled water" is defined as water, which as a result of treatment of waste, is suitable for a direct beneficial use or a controlled use that would not otherwise occur..." Water Code §13050(n). Discharges of recycled water without a permit are prohibited. Water Code §13529.2. A regional board may issue "waste discharge requirements" (*i.e.*, a permit) to an individual discharger. Water Code §13263(a). A regional board may also issue a "master recycling permit" which covers a large number of users and is issued to suppliers or distributors rather than users. Water Code §13523.1(a). Permits must prevent nuisance, which includes anything that is injurious to public health. Water Code §§ 13263(a), 13050(m). Regional Boards may require testing to assess the safety of the discharge. Water Code §13267.

In addition to a regional board's individual permit, the Legislature empowered the Board to adopt "general waste discharge requirements for a category of discharges" under certain circumstances. Water Code §13263(j). General Waste Discharge Requirements ("WDR") are region-wide or state-wide generic permit terms that dischargers can elect to use for permitting their discharges rather than initiating an individual WDR permitting process. Water Code §13263(i). A General WDR does not permit recycled water for potable uses, direct (retail distribution systems) or indirect (groundwater recharge), and rather functioned to permit non-potable sues such as landscape and agricultural irrigation and certain industrial uses such as cooling towers. AR 195.

When adopting a General WDR, the Board must make findings regarding the beneficial uses to be protected, the water quality objectives reasonably required for that purpose, other waste discharges, the need to prevent nuisance, past, present, and probable future beneficial uses, environmental characteristics, water quality conditions that could reasonably be achieved through the coordinated control of all factors which affect water quality in the area, economic considerations, the need for developing housing, and the need to develop and use recycled water. Water Code §§ 13263(a), 13241.

2. CDPH

CDPH is the primary state agency responsible for protection of public health. Opp. RJN Ex. B at 2. Among its public health responsibilities is the regulation of recycled water's impact on public health. CDPH is required to establish statewide recycling criteria for different types of uses of recycled water by setting levels of constituents "which will result in reclaimed water safe

from the standpoint of public health, for the uses to be made." Water Code §§ 13520, 13521.

CDPH has established statewide recycling criteria in California Code of Regulations, title 22, division 4, chapter 3 ("Recycling Criteria"). The Recycling Criteria require that recycled water receive certain levels of treatment, depending on proposed use. Primary stage treatment involves allowing solids to settle to the bottom, which reduces turbidity. Secondary treatment involves oxidation through bubbling air through the water to dissolve oxygen and promote its use by microorganisms that break down organic matter. Tertiary treatment means filtering the water to reduce turbidity to certain standards.

When used for surface irrigation of orchards where the recycled water does not come in contact with edible portions of the crop, recycled water shall be at least "undisinfected secondary recycled water". 22 CCR §60304(d)(1). "[U]ndisinfected secondary recycled water" means oxidized wastewater. 22 CCR §60301.900.

When used for the surface irrigation of food crops where the recycled water comes into contact with the edible portion of the crop, the Recycling Criteria applies the highest standard, which is "disinfected tertiary recycled water." 22 CCR §60304(a)(1). "[D]isinfected tertiary recycled water" means the wastewater has been filtered and subsequently disinfected. 22 CCR §60301.230. To be considered "filtered," the wastewater must be oxidized and filtered to meet specific turbidity standards. 22 CCR §60301.320. The Recycling Criteria permit water to be disinfected using either a chlorine disinfection process or any other disinfection process that has been demonstrated to remove 99.999 percent of polio virus or an equivalent virus. 22 CCR §60301.320(a).

The regulations also require that an engineering report – known as a "title 22 engineering report" -- be prepared by a qualified engineer licensed in California which clearly indicates the means for compliance with these regulations and includes a contingency plan to assure that no untreated or inadequately treated recycled water is delivered. 22 CCR §60323.

D. Statement of Facts[1]

On January 17, 2014, Governor Brown declared a state of emergency due to severe drought conditions. AR 395. The January 2014 Executive Order required a number of conservation activities to decrease water demand, and other actions to increase and reallocate water supply. Id. In order to conserve potable water supplies, the Board's staff began work on encouraging the use of recycled water by streamlining the recycled water permitting process. AR 193.

After three more months of drought, the Governor determined that more emergency actions were necessary. On April 25, 2014, the Governor found that "expedited actions" are needed to mitigate the drought's harmful impacts. AR 5, 396. Among the actions ordered, the Governor directed the Board to "adopt statewide general waste discharge requirements to facilitate the use of treated wastewater [i.e., recycled water] that meets standards set by the Department of Public Health, in order to reduce demand on potable water supplies." AR 397. Because of the emergency

[1] The Board asks the court to judicially notice (1) a 2014 California Water Action Plan issued by the California Environmental Protection Agency, (2) a 1996 Memorandum of Agreement between the Board and CDPH, and (3) portions of the Department of Water Resources 2013 Reliability Report. Petitioner opposes on the grounds that the documents lack relevance. The objection is overruled and the requests for judicial notice are granted. Ev. Code §452(c).

need to implement the expanded use of recycled water in the State and reduce the strain the drought caused for potable water sources, and "to allow these actions to take place as quickly as possible," the Governor declared the Board's actions would be exempt from CEQA. AR 398.

1. The Draft Order

In compliance with the Governor's Executive Order, on April 29, 2014 the Board released for public comment Draft General Waste Discharge Requirements for Recycled Water Use ("Draft Order"). AR 89-124. The Draft order imposed numerous prohibitions on use, such as causing a nuisance. AR 102. It also required compliance with CDPH regulations, preparation of a title 22 engineering report, and other requirements. AR 103. Applicants seeking coverage under the Draft Order were required to submit a notice of intent to enroll, and comply with reporting, notification, education, monitoring and maintenance requirements. AR 104-06.

The Draft Order also stated: "Title 22 imposes limitations on the uses of recycled water, based on the level of treatment and the specific use in order to protect human health. By restricting the use of recycled water to title 22 requirements, this order ensures that recycled water is used safely." AR 97.

Thirty four members of the public submitted written comments, including Petitioner Wilson. AR 451-885.

2. Wilson's Comments

Wilson's written comments argued that the Draft Order would not ensure "that recycled water is used safely" and that recycled water should be tested for perchlorate prior to use as irrigation water for oranges. AR 620-772. Wilson explained that tertiary treated wastewater, which is approved under the Recycling Criteria, may still contain perchlorate, an endocrine-disrupting chemical. Id. He requested that the Board make findings in its Order regarding the levels of perchlorate in the recycled water, the risk that perchlorate could adversely affect the public health if it is present in the water, and the likelihood of adverse effects on the public health due to perchlorate. AR 622.

Attached to Wilson's letter were four articles about perchlorate authored from 2006 to 2012. AR 623. Those articles can be summarized as follows.

Perchlorate reduces "the functioning of the thyroid gland, and poor thyroid function is an important cause of developmental deficits and adult disease." AR 719. In humans, the thyroid gland needs iodide to produce thyroid hormone. Id. A compound known as NIS is responsible for transporting iodide into the thyroid gland. Perchlorate inhibits the ability of NIS to take up iodide. Id. The reduced transport of iodide suppresses the production of thyroid hormone. Id. Thyroid hormone is essential for normal brain development, body growth as well as for adult physiology. AR 719. Recent research indicates that thyroid hormone insufficiency in pregnant women is associated with cognitive deficits in the children. Id.

There is concern that perchlorate-contaminated waters "may represent a health risk both as sources of drinking water and irrigation water for food crops." AR 688. Human exposures to perchlorate "are likely attributed to both contaminated drinking water and food; in fact, a recent analysis concludes that a majority of human exposure to perchlorate comes from food." AR 719.

Perchlorate is not physically or chemically retained by soil (AR 690), and is largely transported into and through soils with irrigation water. AR719. Perchlorate is chemically stable

when wet. AR 719. The California drinking water safety limit for perchlorate is 6 parts per billion. AR 627. Perchlorate can be introduced into municipal sewers from waste discharge by industrial processes using perchloric acid. AR 676-68. Treated municipal wastewater can have perchlorate concentrations ranging from 250 parts per billion to 700 parts per billion. AR 678.

Orange trees can have perchlorate levels that are higher than wastewater. This is because orange trees take up perchlorate with irrigation water, and the concentration in the orange fruit is higher than the concentration in the irrigation water. AR 690. This is because as water taken into a tree evaporates, salts are left behind and accumulate. AR 622. Orange trees in Loma Linda, California, irrigated with contaminated well water with a perchlorate level of 18 parts per billion produced oranges with a perchlorate level of 38 parts per billion. AR 692.

3. The Board Hearing and Order

On June 3, 2014, the Board conducted a public hearing on the Draft Order. AR 163-390. Nine persons presented oral comments at the hearing. AR 199, 231, 240, 270, 273, 279, 283, 293, 300. Wilson did not appear at the hearing. All comments received, including Wilson's, were provided to the Board's members. AR 19.

The Board's staff prepared Change Sheet 2 to revise the Draft Order in response to certain comments. AR 39-43, 322-323. Before rending a final decision, the Board acknowledged that it "heard and considered all comments." AR 17. The Board adopted the Order at the conclusion of the public hearing. AR 1-38.

The Board found specifically that recycled water is safe for approved uses under the Order "[w]hen used in compliance with [the Recycling Criteria], and all applicable state and federal water laws." AR 7. The Order also contains factual findings on the background of the drought, statutory and regulatory issues, degradation of the water supply, and the purpose and applicability of the Order. AR 5-16.

The Order requires that all agencies that intend to be regulated under the Order must comply with certain requirements. AR 17. Among those is a requirement that the use of recycled water must not cause or contribute to pollution or nuisance. Id. The agencies must also comply with the Recycling Criteria, including submitting an engineering report and amendments. AR 18. The Administrator of the permits has the power to discontinue delivery of recycled water if it has reason to believe that the Recycling Criteria are not met. AR 19. Regional water boards are given the ability to terminate a permit under the Order if the use of the permit is endangering the public health or the environment. AR 21.

E. Analysis

Petitioner Wilson seeks a writ of mandate directing the Board to set aside its Order, asserting that (1) the Order is not supported by the findings because there is no finding about perchlorate, and (2) the findings are not supported by substantial evidence because the only perchlorate evidence submitted was Wilson's letter.

1. Adequacy of the Findings

Wilson asserts that the Order contains no findings on perchlorate, and therefore the conclusion that recycled water is safe for approved uses under the Order is not supported. Mot. at 11. The Board, on the other hand, argues that it was only required to make findings regarding the

elements in Water Code section 13263(a), and those findings were made. Opp. at 9-10.

a. The Board's Order is Most Probably Quasi-Legislative

Administrative mandamus provides for judicial review of adjudicatory decisions rendered by *administrative agencies*. Topanga Ass'n for a Scenic Community v. County of Los Angeles, ("Topanga") (1974) 11 Cal.3d 506, 514-15 (emphasis added). It does not establish judicial review of legislative acts. Id. at 816. A legislative act provides what the law shall be in future cases arising under it. Dominey v. Dept. of Pers. Admin., (1988) 205 Cal.App.3d 729, 737 (quoting Union Pac. R. Co. v. U.S. ("Sinking-Fund Cases") (1878) 99 U.S. 700, 761). Actions are legislative in nature when they declare a public purpose and make provisions for the accomplishment of that purpose. O'Loane v. O'Rourke (1965) 231 Cal.App.2d 774, 784-85 (adoption of a general plan by way of a resolution was a legislative act because it prescribed a new policy rather than implementing an existing one).

The Board's Order for a General WDR was a quasi-legislative act because it was a matter of general application for a public purpose -- a general permit for a category of discharges – and not for any specific applicant. *See* AR 9. Because there was no specific applicant, the Board's order was not a quasi-judicial act.

In arguing that the Board's Order was adjudicative in nature, Petitioner relies on the fact that the court's jurisdiction under CCP section 1094.5 ("section 1094.5") for the review of administrative mandamus. Mot. at 9-10. Administrative mandamus is the form of judicial review for challenging a quasi-adjudicatory decision by an agency where the agency is required by law to provide a hearing before issuing a decision. *See* Fukuda v. City of Angels, (1999) 20 Cal.4th 805, 811.

It is true that Water Code section 13263 governs the issuance of waste discharge permits, both individual and general. It is also true that agency action subject to Government Code section 11352 – which refers to the issuance of waste discharge requirements and permits pursuant to Water Code section 13263 – is reviewed under section 1094.5. Water Code §11330(g). Wilson also is correct that Government Code section 11352(b) does not distinguish between individual permits and a General WDR. Mot. at 9-10. Therefore, the Board's issuance of a General WDR arguably is administrative mandamus subject to review under section 1094.5.[2]

Even if it must be reviewed under section 1094.5, the Board's Order was quasi-legislative, not quasi-adjudicative, in nature. Where an agency makes a decision without being required to consider evidence from *opposing* parties, no hearing occurs within the meaning of section 1094.5. 300 DeHaro Street Investors v. Department of Housing and Community Development (2008) 161 Cal.App.4th 1240, 1250. While even purely documentary proceedings can satisfy the hearing requirement of section 1094.5, the agency must be *required by law* to accept and consider evidence from both sides. *See* Friends of the Old Trees v. Department of Forestry & Fire Protection, (1997) 52 Cal.App.4th 1383, 1391. Even then, there must be something in the nature of a hearing -- "an adversarial process in which the agency resolves disputed facts after affording interested parties an opportunity to present evidence." 300 DeHaro, *supra*, 161 Cal.App.4th at 1251.

The fact that the Board's Order was quasi-legislative is important because the requirements

---

[2] The court need not decide whether the Order should be reviewed as traditional mandamus under CCP section 1085.

of Topanga Assn. for a Scenic Community v. County of Los Angeles, ("Topanga") (1974) 11 Cal.3d 506 – that an agency must make sufficient findings to "bridge the analytic gap" between the evidence and the agency's conclusion – only apply to adjudicative decisions, not quasi-legislative decisions. Native Sun/Lyon Communities v. City of Escondido, (1993) 15 Cal.App.4th 892, 910 (findings not required for city's adoption of development plan and development agreement). Moreover, where an agency decision has both adjudicatory and quasi-legislative characteristics, the dominant concern of the action determines whether Topanga requirements will apply. If the dominant concern is narrow and private rather than broad and public, the action is adjudicatory. City of Rancho Palos Verdes v. City Council, (1976) 59 Cal.App.3d 869, 885.

Even if the Board's Order had some adjudicatory characteristics because there will be an unknown number of applicants who enroll, the dominant concern of the Order is broad and public. As a result, the Order is quasi-legislative in nature and Topanga's requirements do not apply even though the judicial review is governed by section 1094.5. This conclusion means that the Board's Order was not required to discuss perchlorate or provide the analytical route in finding that the public health was protected.

In any event, the Board is correct (Opp. at 9) that the Order satisfies Topanga so long as the findings support the conclusion that the legislative requirements for General WDRs set forth in Water Code sections 13263 and 13241 have been satisfied. Topanga, *supra*, 11 Cal.3d at 518. Petitioner Wilson does not dispute that the Order meets these requirements for all issues except public health. He contends consideration of public health is subsumed within the Board's obligation to "take into consideration" "the need to prevent nuisance." Water Code §13263(a). A "nuisance" includes anything injurious to health. Water Code §13050(m). Reply at 6-7.

The court accepts that the Board has an obligation to consider the need to protect public health in issuing a General WDR. As Wilson points out (Reply at 8), the Board acknowledged this obligation in its Order, and did not shirk from this duty at the hearing. However, the Board found as part of the Order that CDPH has primary responsibility for protecting public health in the level of treatment, disinfection, potential for public contact, and use of recycled water, and CDPH has issued the Recycling Criteria for that purpose. AR 6-7. The Board's Order found that recycled water is safe when used in compliance with the Recycling Criteria. AR 7.

While this finding does not specifically address perchlorate, there is no requirement that the Board's findings address all of the evidence submitted. Unlike CEQA, there is no statutory requirement for issuance of General WDRs that the Board respond be made to all public comments. *See* Pub. Res. Code §21091(d)(2). Such a requirement would be impractical since the Board received hundreds of pages of written comments and hours of public comment at the hearing. The Board is only required to consider the evidence presented, which it did. AR 17.

In sum, Water Code section 13263(a) requires that the Board take into consideration the need to prevent nuisance. Water Code §13263(a). A nuisance includes anything that is injurious to health. Water Code §13050(m). In issuing the Order, the Board found that the public's health would be protected by the Recycling Criteria. AR 7. The Board's finding is sufficient to satisfy the statutory requirement that it consider the need to prevent nuisance.

2. Substantial Evidence to Support the Order

Wilson argues that the Board's finding that recycled water is safe when used in compliance with the Recycling Criteria is not supported by substantial evidence because his written comment

and the four articles he submitted were the only evidence on perchlorate levels. Mot. at 12-13.

The record contains no evidence contradicting Wilson's submitted articles regarding the existence and significance for humans of perchlorate in recycled water. However, this does not mean that the Board's decision was not based on substantial evidence. CDPH has the primary statewide responsibility for protecting public health. AR 6. CDPH has determined what uses are safe for different types of recycled water and at what levels of contaminants. 22 CCR §60301.900. This includes the use of secondary recycled water to irrigate orchards and tertiary recycled water where it directly contacts food crops. 22 CCR §60304. The Board has consistently relied on CDHP's expertise in the establishment of conditions needed to protect public health. AR 409. The Board's finding that recycled water is safe for approved uses is based on CDPH's Recycling Criteria. The regulations of an agency with appropriate expertise can provide substantial evidence in support of another agency's decision. Oakland Heritage Alliance v. City of Oakland. ("Oakland") (2011) 195 Cal.App.4th 884, 903-04.

Wilson replies that 22 CCR section 60304 ("section 60304") was issued 15 years ago and does not specifically mention perchlorate. His articles on perchlorate are much more recent. He notes that the Board's opposition does not dispute that perchlorate levels are not reduced by tertiary recycled water, and it is not reasonable to conclude that the CDPH regulation protects humans against perchlorate. Reply at 2-3, 9-10.

This is an issue of substantial evidence. The Board is not prohibited from accepting the expertise of the CDPH merely because the Board has been given oversight of recycled water issues. The question is whether the agency that made the regulations has the requisite expertise, which CDPH clearly does. CDPH's Recycling Criteria constitutes substantial evidence in support of the Board's decision. "Substantial evidence" is relevant evidence that a reasonable mind might accept as adequate to support a conclusion. California Youth Authority v. State Personnel Board, (2002) 104 Cal.App.4th 575, 585. The Recycling Criteria set forth specific requirements as to the different types of recycled water that are appropriate for various uses.

Wilson attempts to distinguish Oakland by noting that it concerned a city's approval of a development project and its finding that seismic mitigations were adequate because they were based in part on compliance with the Building Code. The appellate court concluded that compliance with the Building Code, which was intended to promote structural safety in the event of an earthquake, was substantial evidence that the project's seismic risk was less than significant. 195 Cal.App.4th at 904. Wilson argues that, unlike CDPH's regulation which does not mention perchlorate, the Building Code addresses the specific seismic risk for structures that was at issue in Oakland. Reply at 3-4.

The premise of this purported distinction is false. Wilson assumes that section 60304 does not protect against perchlorate because the regulation does not expressly mention it. But the provision does not expressly mention any recycled water contaminant. Rather, the regulation is written in a manner that requires particular recycled water treatments for orchard irrigation, and for irrigation of food crops where the water contacts edible portions of the crop. The regulation addresses the contaminants in recycled water that could affect humans, and the treatments are intended to protect public health from adverse impacts. An agency is presumed to have regularly performed its official duties. Ev. Code §664. CDPH must be presumed to have done its job in issuing the regulation and considered all potential contaminants and uses of recycled water, including perchlorate contamination. Wilson has not met his burden of showing otherwise. *See*

Steele, *supra*, 166 Cal.App.2d at 137. For example, he has not shown that the articles he submitted purport to make new findings about perchlorate that were unknown at the time section 60304 was promulgated.[3]

Finally, the Board correctly points out that the Governor directed it to adopt General WDRs "that meet standards set by" CDPH. AR 397 ¶10. The Board can hardly be blamed for relying on CDPH standards in making its finding. If the Board's finding is wrong because the CDPH standards are outdated, Wilson's remedy is a petition to CDPH to amend or repeal section 60304.

F. Conclusion

Wilson presented evidence to the Board of a threat to the public health from perchlorate in the use of recycled water in orange groves, and the Board, after reviewing all of the evidence, found that following CDPH's Recycling Criteria would adequately protect the public health.

The petition for writ of mandate is denied. The Board's counsel is ordered to prepare a proposed judgment, serve it on Petitioner's counsel for approval as to form, wait 10 days after service for any objections, meet and confer if there are objections, and then submit the proposed judgment along with a declaration stating the existence/non-existence of any unresolved objections. An OSC re: judgment is set for August 27, 2015.

[3] Neither party has attached the pertinent regulations or provided the amendment history for 22 CCR section 60304.

EXHIBIT 7

ANDREW C. WILSON
State Bar No. 133062
7468 Dufferin Avenue
Riverside, CA 92504
Telephone: (951) 687-4471
E-mail: acwilson11@yahoo.com

Petitioner In Pro Se

SUPERIOR COURT OF THE STATE OF CALIFORNIA

COUNTY OF LOS ANGELES

CENTRAL DISTRICT

ANDREW C. WILSON, Petitioner, **v.** **STATE WATER RESOURCES CONTROL BOARD,** Respondent.	Case No. BS149632 **PETITIONER'S MEMORANDUM OF POINTS AND AUTHORITIES IN SUPPORT OF MOTION FOR NEW TRIAL** Dept: 85 Judge: The Honorable James C. Chalfant Hearing Date: October 15, 2015 9:30 a.m. Action Filed: July 3, 2014

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Cases

Statutes

Regulations

Other

I. INTRODUCTION

In this case Petitioner challenges a general permit adopted by Respondent (the Board) that allows recycled water to be used for irrigating all crops grown in California. The permit requires compliance with safety regulations (referred to as "title 22") that have been adopted by the California Department of Public Health (CDPH). Although the permit requires compliance with title 22, Petitioner contends that a reasonable person would take further precautions, and would test recycled water for perchlorate before using it to irrigate oranges.

At the hearing in this case Petitioner was surprised that the Court relied on Evidence Code section 664, which provides: "It is presumed that official duty has been regularly performed." The Court applied the presumption to the title 22 regulations adopted by the CDPH. The Board had never raised the issue. Petitioner never considered that the presumption applied and was not prepared to argue the point.

The Court's decision that no findings were required even though Code of Civil Procedure section 1094.5 governs review also caught Petitioner off guard. Both parties in their briefs had taken the position that findings were required.

In this motion Petitioner seeks clarification of the procedural duties of the Board. Petitioner presents new legal arguments that he did not previously have a chance to present to the Court. The Court's decision on proper procedures affects public health and has enormous statewide importance. The Court's decision on this motion for new trial not only affects Petitioner, but, more importantly, it will affect every person who comes after Petitioner. Petitioner urges the Court to take another look at this case because this case is a case of first impression and has wide-ranging implications for public health.

II. SUMMARY OF RELIEF REQUESTED

Petitioner requests that the Court enter a conditional order granting this motion for new trial. As explained below, Petitioner requests that the Court enter an order providing that this motion is *granted* unless the Board consents to entry of a modified judgment on or before October 16, 2015, and in the event of the Board's timely consent, then this motion for new trial is *denied.*

Petitioner's proposed modified judgment does not set aside the general permit. Instead, it requires that the Board submit Petitioner's comment to the California Department of Public Health (CDPH) and request that the CDPH provide its written views and recommendations to the Board. After receiving the CDPH's views, the Board is required to exercise its sound discretion and revise the general permit as the Board may deem necessary as provided in paragraph D.6 of the permit, which states: "The State Board will review this General Order periodically and may revise the requirements as deemed necessary." (AR 22) Petitioner's proposed modified judgment is attached hereto as Exhibit A.

III. FACTS

Petitioner Andrew C. Wilson (Petitioner) commenced this action by filing a petition seeking a writ of administrative mandate ordering Respondent State Water Resources Control Board (the Board) to set aside the order adopted on June 3, 2014, entitled "General Waste Discharge Requirements for Recycled Water Use" (the Order).

The Order is known as a general permit. The Board issued the general permit pursuant to the provisions of the Porter-Cologne Water Quality Control Act (the Act). (Water Code §§ 13000 et seq.)

Two agencies have responsibilities for regulating recycled water under the Act: The Board and the CDPH. (Water Code §§ 13001, 13520, 13521; AR 408-409.) Prior to issuing the general permit, the Board held a public meeting to receive comments, and Petitioner submitted a comment consisting of a letter and four scientific articles to oppose the issuance of the permit. (AR 620.) Petitioner's comment presented evidence on an issue of public health: Whether a reasonable person would take precautions to prevent harm to public health beyond what is required by title 22.

The record contains no evidence that Petitioner's comment was submitted to the CDPH for review and recommendations or that the Board's decisionmakers received the CDPH's views or recommendations on Petitioner's comment into evidence. The Board found that use of recycled water in compliance with the CDPH's regulations is safe. (AR 7.)

On review, this Court upheld the permit and denied the petition based on a written

decision (the Decision). (Declaration of Andrew C. Wilson, Ex. A.) On August 17, 2015, the Court rendered a judgment denying the petition. (Declaration of Andrew C. Wilson, Ex. B.) A copy of the Reporter's Transcript (RT) for the trial hearing is attached as Exhibit C to the Declaration of Andrew C. Wilson.

Petitioner brings this motion for new trial on the following grounds: (1) insufficiency of the evidence to justify the decision, (2) the decision is against law, and (3) error in law, occurring at the trial and excepted to by the party making the application.

IV. JURISDICTION

This Court has authority to grant a motion for new trial in this case. New trial procedures "apply in proceedings for administrative mandamus brought pursuant to section 1094.5." (*Pollack v. State Personnel Board* (2001) 88 Cal.App.4th 1394, 1405 [107 Cal.Rptr.2d 39].) The "courts have long recognized that motions for new trial may be made in proceedings for administrative mandamus brought pursuant to section 1094.5." (*Id.*)

Petitions for administrative mandamus brought pursuant to section 1094.5 may involve only issues of law. A new trial may be granted in cases involving only issues of law, even though Code of Civil Procedure § 656 defines a new trial as: "A new trial is a re-examination of an issue of fact in the same court after a trial and decision by a jury, court, or referee." At one time appellate courts relied on section 656 to hold that new trials could not be granted if only issues of law and no issues of fact had been tried, but the Supreme Court disapproved those cases in *Carney v. Simmonds* (1957) 49 Cal.2d 84 [315 P.2d 305]. The Supreme Court stated that section 656 must be "read and construed in conjunction with the basic section on motions for new trial, section 657 of the Code of Civil Procedure." (*Carney v. Simmonds*, *supra*, 49 Cal.2d at p. 90.) Section 657 provides that grounds for new trial include that the "decision is against law" and "error in law occurring at trial." These grounds show that trial courts have authority to grant new trials even though only issues of law were decided. The Supreme Court held: "As a matter of orderly procedure there is no less reason why the trial court should have a second chance to reexamine its judgment where issues of fact are involved than where issues of law or law and fact are decided." (*Id.*)

The Clerk of the Court mailed notice of entry of judgment to the parties on August 17, 2015. Petitioner timely served and filed a notice of intention to move for new trial on September 1, 2015, within 15 days of the day the Clerk mailed notice of entry of judgment.

V. SUMMARY OF NEW TRIAL PROCEDURE

The Court must hear a motion for new trial within 60 days from the date the Clerk mails notice of entry of judgment pursuant to Code of Civil Procedure section 664.5. (Code of Civil Procedure § 660.) The 60 day time limit is jurisdictional. After 60 days the trial court has no jurisdiction to grant the motion. Motions not heard within the 60 days are deemed denied. (*Id.*)

An order granting a motion for new trial must be in writing and must (1) state the grounds for granting the motion, and (2) provide a specification of reasons for granting the motion upon each ground stated. (Code of Civil Procedure § 657.) A Court may not direct the attorneys to prepare an order granting a new trial. (*Id.*)

A motion for new trial may be granted on the basis of legal arguments previously rejected (error in law occurring at trial), or on the ground of entirely new legal theories and arguments (decision is against law). (*Hoffman-Haag v. Transamerica Ins. Co.* (1991) 1 Cal.App.4th10, 15 [1 Cal.Rptr.2d 805].)

The Court has the power to impose terms and conditions in orders granting or denying new trial motions. "Such conditions may require one party or the other to consent to a modification of the judgment or perform some other act in order to avoid, or obtain, a new trial." (Wegner, et al. Cal. Prac. Guide: Civil Trials & Evidence § 18:438, p. 18-108 (Rutter Group 2014).) "It is standard practice in California for trial courts to impose reasonable terms and conditions on granting or denying motions for new trials in actions tried by a jury as well as those tried by a court." (*Chapman v. Municipal Court* (1949) 91 Cal.App.2d 689, 691 [205 P.2d 712].)

VI. ARGUMENT

A. The Board Had A Duty To Consult With The CDPH On Petitioner's Comment Because It Is Possible The CDPH Shares Petitioner's View of Dangers to Public Health.

The general permit the Board adopted lacks evidentiary support because the administrative record contains no evidence showing the current views of the CDPH on the

questions of public health Petitioner raised. This argument is a new argument that Petitioner has not previously presented to the Court.

The only way to protect the public health is for this Court to require the Board to consult with the CDPH whenever the evidence shows that the answer to a public health question is not clear-cut, and it is possible for reasonable minds to differ. Otherwise, if the Board does not check with the CDPH, there is a possibility that the CDPH agrees with Petitioner and public health will be put in jeopardy.

An ongoing duty of inter-agency collaboration is implicit in the statutory scheme because collaboration is essential to ensure the maximum protection of public health. Statutes must be construed in a manner that makes them reasonable and fulfills the statutory purpose. "[C]ivil statutes for the protection of the public are, generally, broadly construed in favor of that protective purpose." (*People ex rel. Lundgren v. Superior Court* (1996) 14 Cal.4th 294, 313.)

To satisfy the duty of collaboration, the Board's decisionmakers, when deciding public health questions at a hearing, must consider evidence of the current views of the CDPH on public health questions that are open to reasonable difference of opinion. If the hearing record lacks such evidence, the Board's decision must be set aside for lack of evidentiary support.

The views of the CDPH must be received in evidence because the Board's decisionmakers must base their decision on the evidence presented at the hearing.

The duty of collaboration does not extend to all public health questions. There is no duty of collaboration if the evidence in the record shows with certainty that the CDPH would not agree with the position that a commenter has taken.

The Legislature and the Board and the CDPH recognize the need for ongoing and continuous inter-agency collaboration on issues of public health.

Water Code sections 13523 and 13523.1 require regional boards to consult with and receive the recommendations of the CDPH prior to issuing permits allowing recycled water use.

In 1996, the two agencies executed the "Memorandum of Agreement" (MOA) delineating their respective obligations. The MOA states that the CDPH is "the primary State agency responsible for protection of public health." (MOA, p. 2 (a copy of the MOA is attached as Ex. A

to the Declaration of Andrew C. Wilson).)

The state's Recycled Water Policy adopted by the Board provides: "Regional Water Boards *shall* appropriately rely on the expertise of CDPH for the establishment of permit conditions needed to protect human health." (AR 409, italics added.)) The Board in its Opposition Brief states: "Indeed, the State Water Board had consistently relied 'on the expertise of CDPH for establishment of permit conditions needed to protect human health' (AR 409)." (Opposition Brief, p. 12.)

The Board's Order at issue in this case states: "[T]he MOA allocates primary areas of responsibility and authority between these agencies, and provides for methods and mechanisms necessary to ensure *ongoing, continuous future* coordination of activities relative to the use of recycled water in California." (AR 6, italics added.)

The MOA states: "The RWQCBs will defer to the Department with respect to *any question* involving interpretation of any Title 22 criteria." (MOA p. 7, italics added.) The MOA states: "In the process of issuing reclamation requirements, the RWQCBs must consult with and consider the recommendations of the Department (Water Code Section 13523)." (MOA p. 2)

The foregoing provisions of statutes, policies, orders and the MOA collectively show recognition of the necessity of ongoing inter-agency collaboration. Proper construction of the statutory scheme as a whole recognizes a duty of collaboration on public health questions that are open to reasonable difference of opinion.

B. <u>The Record Contains No Evidence Showing That the Board Fulfilled Its Duty To Consider the CDPH's Views on Petitioner's Comment</u>.

In the instant case, the Court invoked the presumption of Evidence Code section 664, which provides: "It is presumed that official duty has been regularly performed." Presumptions are "not 'evidence' but are conclusions that *the law requires to be drawn (in the absence of a sufficient contrary showing)* when some fact is proved or otherwise established in the action." (Evidence Code § 600 Comment – Assembly Committee on Judiciary, italics added.)

The Court held that in order for Petitioner to meet his burden, Petitioner had to show that "the articles he submitted purport to make new findings about perchlorate that were unknown at

the time section 60304 was promulgated." (Decision, p. 10.) At the hearing the Court stated: "[Petitioner's] articles are more recent, the implication being that the more recent articles give new-found information. Well, you haven't shown that." (RT 2-3.) The Court further stated: "[I]f you had shown *that here is what the Department of Public Health had before it when it made its ruling on perchlorate* and here is what I have now and, boy, this is a much more serious risk to public health than the Board thought, than the Department of Public Health thought, then you might be in a different situation. Of course, *you would have had to present that to the Water Board.*" (RT 15, italics added.)

Petitioner's burden, as described by the Court, is an impossibly heavy one. According to the Court's decision, the Board must reject the arguments of a person questioning the sufficiency of title 22 if the person fails to meet that burden. However, it is absurd to imagine that an ordinary person would include with his comments evidence of what the CDPH had before it when it adopted title 22. The Court's decision paves the way for the Board to reject virtually all public comments questioning the sufficiency of title 22, including those comments presenting information of a contaminant that the CDPH overlooked or a contaminant that the CDPH would want to re-evaluate. The Court's decision *requires* the Board's decisionmakers to make decisions that potentially conflict with the views of the CDPH and that put the public health at risk.

In the instant case, it would be possible for the CDPH to conclude, based on the evidence in the administrative record, that a reasonable person would take additional precautions to prevent harm to public health beyond the requirements of title 22.

Petitioner submitted evidence that title 22 treatments do not remove perchlorate. The regulation imposes no numeric limits for perchlorate in recycled water; unlimited amounts of perchlorate are allowed. (Copies of pertinent provisions of title 22 are attached as Exhibit E to the Declaration of Andrew C. Wilson.)

Crop irrigation is governed by section 60304 of title 22. It is undisputed that section 60304 was last revised in the year 2000. (Petitioner requests the Court to take judicial notice of the amendment history set forth in Exhibit E, p. 605 to the Declaration of Andrew C. Wilson.)

Petitioner submitted factual data that did not exist when the CDPH last revised section

60304 in the year 2000. Petitioner's factual data included the following. In 2004-2005 data was collected at Loma Linda, California showing that orange trees take up perchlorate with irrigation water, and the concentration in the fruit is *higher* than the concentration in the irrigation water (water with a perchlorate level of 18 parts per billion produced oranges with a perchlorate level of 38 parts per billion). (AR 689) In 2004 data was collected in Massachusetts showing that perchlorate concentrations in secondary treated municipal wastewater can be as high as 250 to 700 parts per billion. (AR 677-678) In 2006 a scientific article was published showing a method of estimating the exposure from eating oranges contaminated with perchlorate and comparing it to the no-effect reference dose recommended by the National Academy of Sciences. (AR 692) The method applied to hypothetical oranges with a perchlorate level of 250 parts per billion results in estimated adult exposure exceeding the no-effect reference dose recommended by the National Academy of Sciences, and results in child exposure almost four times the recommended no-effect reference dose. (AR 692, Petitioner's Opening Brief, p. 5 n. 3.) A scientific article published in 2011 found that data collected in 2001-2002 suggests that the thyroid function in adult women is affected by lower exposures than the no-effect reference dose recommended by the National Academy of Sciences. (AR 403.) In 2012 a scientific article was published stating: "Recent work [published in 2011] indicates that very subtle thyroid hormone insufficiency in pregnant women is associated with cognitive deficits in their children." (AR 719, 759.)

Based on those articles, Petitioner claimed that mere compliance with title 22 is not sufficient to protect the public health. In order to prevent harm to the public health, a reasonable person would take further precautions and would test disinfected tertiary recycled water for perchlorate before using the water to irrigate oranges. Petitioner claimed that it would not be sufficient to rely on speculative argument that there is no perchlorate in California recycled water. Petitioner's comment stated: "Testing water for perchlorate is not expensive. Rather than speculating or arguing that perchlorate levels are likely to be low, or likely to be high, the levels should simply be tested." (AR 621.) The test results would be used to guide further action. For example, if the test showed no perchlorate, or perchlorate below a CDPH approved safety level for the crop in question, then use of the recycled water would be allowed. If the test results would

cause a reasonable person to recognize that the safety of the water cannot be determined without further study or investigation, then the water must not be used pending the outcome of further investigation.

The question presented was: In light of Petitioner's evidence, would a reasonable person, in order to prevent harm to the public health, take further precautions and test disinfected tertiary recycled water for perchlorate before using the water to irrigate oranges?

It would be possible for the CDPH to conclude, based on the evidence in the record, that a reasonable person would take those precautions. The Board's decisionmakers should not have decided the matter without receiving and considering evidence of the CDPH's current views on the question. The record contains no evidence showing that the Board fulfilled its duty to consider evidence of the CDPH's current views.

C. <u>The Court's Application of Evidence Code 664 Assumes Facts Not In Evidence</u>.

The Court decided that the CDPH has an official duty to consider all potential contaminants and uses of recycled water, including perchlorate, prior to issuing title 22 regulations, and to issue regulations that protect the public health from all adverse impacts. The Court assumes the existence of the official duty, but alleged duty is not supported by evidence in the record. The Court did not consult with the CDPH in arriving at its determination, and there is no evidence in the administrative record showing that the CDPH agrees with the Court.

A presumption is "an assumption of fact that the law requires to be made from *another fact or group of facts found or otherwise established in the action.* A presumption is not evidence." (Evidence Code § 600(a), italics added.) The fact "found or otherwise established in the action" is known as the "basic" fact.

Evidence Code section 664 provides: "It is presumed that official duty has been regularly performed." The "basic" fact giving rise to the presumption of Evidence Code 664 is the existence of an official duty. In order to give rise to the presumption, there must be a legal duty upon a public official to act in a certain way. "Evidence Code section 664's presumption applies *only* where a person has an 'official duty' to perform an act." (*Furman v. Department of Motor Vehicles* (2002)100 Cal.App.4th 416, 422 [122 Cal.Rptr.2d 520], italics added.) In *Furman* the

party invoking the presumption of section 664 provided no foundational evidence that could have supported a finding that an alleged "official duty" existed. (*Id.* at p. 422.) The Court held that the absence of an official duty precludes the application of Evidence Code section 664's presumption. (*Id.* at p. 423.)

Not all contaminants are amenable to statewide recycling criteria. In this case Petitioner identified a health risk posed by perchlorate that may be described as a "systemic" risk. The risk is systemic because it involves a dangerous substance in the irrigation water being taken up through the roots and concentrating *inside* the fruit. Systemic risk is highly variable because it depends on climate and the crop species. (AR 622.) As water evaporates from a plant into the air, chemicals that have been taken up in the plant with the irrigation water are left behind and accumulate in the plant. (*Id.*) The water demand and uptake of a given plant species depends on climate, and is much higher in desert regions of the state than in the cooler coastal plains. For this reason the accumulation of chemical constituents in plants is lower in the coastal regions. (*Id.*)

A statewide standard protecting against systemic risks would necessarily be wasteful. A standard that is strict enough to make desert-grown crops safe will be stricter than needed near the coast. Such a standard would prohibit the use of recycled water near the coast that would otherwise be perfectly safe, and would tend to defeat the state's goals of increased recycled water use.

For these reasons, it is possible that the CDPH, in the exercise of its discretion, could reasonably conclude based on evidence that it should not impose statewide criteria for systemic risks. The CDPH could reasonably decide that systemic risk is more appropriately addressed by local or regional recycling criteria rather than statewide criteria, and could exclude systemic risk from the scope of the title 22 statewide criteria. Following that decision there would be no justification to commit the resources and time to evaluate all systemic risks before issuing title 22 regulations.

A decision to exclude protection against systemic risk would not violate the CDPH's enabling statute. The CDPH's enabling statute for title 22 regulations is Water Code section 13521, which provides: "The State Department of Public Health shall establish uniform

statewide recycling criteria for each varying type of use of recycled water where the use involves the protection of public health." (Italics added.) Recycling criteria are defined as: "As used in this article 'recycling criteria' are the levels of constituents of recycled water, and means for assurance of reliability under the design concept which will result in recycled water safe from the standpoint of public health, for the uses to be made." (Water Code § 13520.)

"Statewide" recycling criteria are different from "regional" or "local" recycling criteria. The enabling statute is broadly drawn and does not dictate which risks are properly addressed in statewide criteria as opposed to regional or local criteria. The statute leaves it to the discretion of the CDPH to decide on the content of its regulations, and to decide, based on evidence, that protection against systemic risk is properly excluded from such regulations. In other words, the enabling statute imposes no legal duty requiring the CDPH to include protection against systemic risk in its title 22 statewide regulations.

This Court lacks authority to declare, purely as a matter of law, that the CDPH is under a legal duty to include protection against systemic risk in its title 22 regulations. The Court's ruling is an unreasonable construction of the enabling statute that improperly limits the CDPH's discretion.

The Court's application of section 664 assumes facts not in evidence. Specifically, the Court assumes the existence of an official duty of the CDPH to include protection against systemic risks in the title 22 statewide regulations. The existence of the claimed official duty is not a fact found or otherwise established in the action. It is not a fact supported by substantial evidence in the record. The Court did not consult with the CDPH about the alleged official duty, and there is no evidence in the administrative record showing that the CDPH agrees with the Court. The motion for new trial is properly granted on the ground of "insufficiency of the evidence to justify the [Court's] decision." (Code of Civil Procedure § 657(6).)

D. <u>The Court's Decision That Findings Are Not Required Conflicts With *Topanga*</u>.

The Court has declared that general permits need not be supported by findings even though review is governed by Code of Civil Procedure section 1094.5. The Court's Decision at

page 8 states: "Even if the Board's Order has some adjudicatory characteristics because there will be an unknown number of applicants who enroll, the dominant concern of the Order is broad and public. *As a result, the Order is quasi-legislative in nature and Topanga's requirements do not apply even though judicial review is governed by section 1094.5.*" (Italics added.)

Water Code section 13330 is an independent legislative mandate to apply section 1094.5 in this case. At the hearing the Court stated: "I agree with Mr. Wilson that the statute says 1094.5 applies . . ." (RT 2) The Court stated: "It's clearly 1094.5, governed by 1094.5." (RT 5). [1]

The quasi-legislative/quasi-judicial analysis is not germane when there exists an independent legislative mandate to apply 1094.5. The analysis is relevant only when there is no independent mandate, and then the analysis is used to determine whether to apply 1094.5.

There is nothing inherently wrong or unconstitutional if a legislature creates an agency and requires that agency to make findings in proceedings that have both adjudicatory and legislative characteristics. For example, one of the procedural models for rulemaking in the federal Administrative Procedure Act requires not only findings, but also an opportunity for cross-examination of witnesses. Congress has the authority to impose this rulemaking model on agencies it creates. (*see United States v. Florida East Coast Ry.* (1973) 410 U.S. 224, 234-238 [35 L.Ed.2d 223, 93 S.Ct. 810].)

Once it is established that 1094.5 applies, either by virtue of an independent legislative mandate, or as a result of a quasi-legislative/quasi-judicial analysis, then the case is governed by

---

[1] Water Code sections 13330(a) and (e) provide that an "order" of the Board is reviewed under section 1094.5. Water Code § 13330(g) provides that an "order" of the Board includes agency action subject to Section 11352 of the Government Code. Agency action subject to section 11352 of the Government Code includes:

> "The issuance, denial, or revocation of *waste discharge requirements and permits pursuant to Sections 13263 and 13377 of the Water Code* and waivers issued pursuant to Section 13269 of the Water Code." (Gov. Code § 11352(b), italics added.)

The above-quoted language refers to "waste discharge requirements" issued pursuant to Section 13263 without distinguishing between "individual" or "general" waste discharge requirements. Both types are issued pursuant to Section 13263 (and Section 13377). Since the language of Government Code Section 11352 does not make any distinction, or carve out any sub-category of waste discharge requirements, the language is properly construed to include both types of waste discharge requirements.

the Supreme Court decision in *Topanga Assn. for a Scenic Community v. County of Los Angeles* (1974) 11 Cal.3d 506 [113 Cal.Rptr. 836].

Petitioner seeks to have the Court's decision that findings are not required set aside on the ground of legal error. Petitioner is entitled to have that erroneous decision set aside even though the Court also ruled in the alternative that if findings were required, then the Board satisfied that requirement in this case.

The erroneous decision adversely affects Petitioner's rights in his future dealings with the Board. The decision fundamentally changes how Board hearings will be conducted. As the Supreme Court explained: "Among other functions, a findings requirement serves to conduce the administrative body to draw legally relevant sub-conclusions supportive of its ultimate decision; the intended effect is to facilitate orderly analysis and minimize the likelihood that the agency will randomly leap from evidence to conclusions. . . . [Findings] also serve a public relations function by helping to persuade the parties that administrative decision-making is careful, reasoned, and equitable." (*Id.*, at pp. 516-517.)

The erroneous decision adversely affects the legal rights of everyone who participates in future general permit hearings before the State Board or the Regional Boards. The right to require the Board to make findings is a significant right. Eliminating that right results in substantial injury to Petitioner and to the public right.

VII. REQUESTED RELIEF

Petitioner requests that the Court render an order on this motion providing as follows:

Petitioner has proposed a modified judgment, a copy of which has been served on Respondent and is attached as Exhibit A to Petitioner's Memorandum of Points and Authorities in Support of Motion for New Trial (the Proposed Judgment). Petitioner's motion for new trial is GRANTED unless Respondent consents to entry of the Proposed Judgment on or before October 16, 2015, and in the event of Respondent's timely consent, then this motion for new trial is DENIED. Respondent's consent to entry of the Proposed Judgment may be stated on the record in open Court, or consent may be given by serving and filing a written notice of consent. The motion for new trial is granted on the following

grounds: (1) insufficiency of the evidence to justify the decision, (2) the decision is against law, and (3) error in law, occurring at the trial and excepted to by the party making the application. The motion for new trial is granted for the following reasons.

1. There is insufficient evidence showing (a) that Petitioner's comment was submitted to the CDPH for review and recommendations, and (b) that the Board's decisionmakers received the CDPH's views or recommendations on Petitioner's comment into evidence. There is insufficient evidence showing that the CDPH had an official duty to include protection against systemic risks in the title 22 statewide regulations.

2. The decision is against law because the Board failed to fulfill its duty to consult with and receive the recommendations of the CDPH on questions of public health that are open to reasonable difference of opinion, specifically, the question presented by Petitioner's comment.

3. The decision that no findings are required was error in law, occurring at the trial and excepted to by Petitioner.

VIII. CONCLUSION

For all the foregoing reasons, Petitioner respectfully urges the Court to grant the requested relief.

Dated: September 11, 2015

Respectfully submitted,

ANDREW C. WILSON

Andrew C. Wilson

Andrew C. Wilson
Petitioner In pro se

EXHIBIT A

SUPERIOR COURT OF THE STATE OF CALIFORNIA

COUNTY OF LOS ANGELES

CENTRAL DISTRICT

ANDREW C. WILSON, Petitioner, **v.** **STATE WATER RESOURCES CONTROL BOARD,** Respondent.	Case No. BS149632 **[PROPOSED] JUDGMENT** Dept: 85 Judge: The Honorable James C. Chalfant Action Filed: July 3, 2014

On July 3, 2014, Petitioner Andrew C. Wilson ("Petitioner") commenced this action by filing a Petition For Writ of Mandate ("Petition") against Respondent State Water Resources Control Board ("Respondent"). On May 27, 2014, Petitioner submitted to Respondent a letter dated May 27, 2014, and four scientific articles ("Petitioner's Comment"). On June 3, 2014, Respondent adopted an order entitled "General Waste Discharge Requirements for Recycled Water Use" (the "General Order"). On August 17, 2015, a judgment (the "8-17-15 Judgment") was entered in this action based on a written decision (the "Decision") rendered by the Court. The Court has rendered a conditional order granting a motion for new trial brought by Petitioner. Both parties have consented to entry of this Judgment.

NOW THEREFORE IT IS HEREBY ORDERED, ADJUDGED AND DECREED that:

1. The 8-17-15 Judgment is vacated. The Decision is set aside to the extent it conflicts with this Judgment.

2. The Court declares that:

a. When issuing general waste discharge requirements pursuant to Water Code section 13263, Respondent has a duty to consult with and receive the recommendations of the California Department of Public Health (the "CDPH") on questions of public health that are open to reasonable difference of opinion.

b. The Supreme Court decision in *Topanga Assn. for a Scenic Community v. County of Los Angeles* (1974) 11 Cal.3d 506 applies to general waste discharge requirements issued pursuant to Water Code section 13263.

3. Respondent shall submit a copy of this Judgment and Petitioner's Comment to the CDPH and request that the CDPH provide a written statement of the agency's official views on the following question: Whether a reasonable person, in order to prevent harm to the public health, would test disinfected tertiary recycled water for perchlorate before using the water to irrigate oranges. The CDPH may consult with Petitioner when considering that question. Respondent shall provide a copy of the CDPH's written views to Petitioner upon receipt. After receiving the CDPH's written views, Respondent shall exercise its sound discretion and revise the General Order as Respondent may deem necessary as provided in paragraph D.6 of the General Order, which provides: "The State Board will review this General Order periodically and may revise the requirements as deemed necessary."

4. Petitioner's Petition is denied. Petitioner takes nothing by way of his Petition.

5. Judgment is entered in favor of Respondent and against Petitioner.

6. Neither party is the prevailing party for purposes of recovering costs.

Dated: _____, 2015

_____
The Hon. James C. Chalfant
Judge of the Superior Court

POS-030

ATTORNEY OR PARTY WITHOUT ATTORNEY (Name, State Bar number, and address):	FOR COURT USE ONLY
ANDREW C. WILSON STATE BAR NO. 133062 7468 Dufferin Avenue Riverside, CA 92504 TELEPHONE NO.: (951) 687-4471 FAX NO. (Optional): E-MAIL ADDRESS (Optional): acwilson11@yahoo.com ATTORNEY FOR (Name): In pro se	
SUPERIOR COURT OF CALIFORNIA, COUNTY OF Los Angeles STREET ADDRESS: 111 North Hill Street MAILING ADDRESS: CITY AND ZIP CODE: Los Angeles, CA 90012 BRANCH NAME: Central District - Stanley Mosk Courthouse	
PETITIONER/PLAINTIFF: ANDREW C. WILSON RESPONDENT/DEFENDANT: STATE WATER RESOURCES CONTROL BOARD	
PROOF OF SERVICE BY FIRST-CLASS MAIL—CIVIL	CASE NUMBER BS149632

(Do not use this Proof of Service to show service of a Summons and Complaint.)

1. I am over 18 years of age and **not a party to this action.** I am a resident of or employed in the county where the mailing took place.

2. My residence or business address is:
   7468 Dufferin Avenue
   Riverside, CA 92504
3. On *(date)*: September 11, 2015 I mailed from *(city and state)*: Riverside, California
   the following **documents** *(specify)*:
   Petitioner's Memorandum of Points and Authorities in Support of Motion For New Trial

   ☐ The documents are listed in the *Attachment to Proof of Service by First-Class Mail—Civil (Documents Served)* (form POS-030(D)).
4. I served the documents by enclosing them in an envelope and *(check one)*:
   a. ☑ **depositing** the sealed envelope with the United States Postal Service with the postage fully prepaid.
   b. ☐ **placing** the envelope for collection and mailing following our ordinary business practices. I am readily familiar with this business's practice for collecting and processing correspondence for mailing. On the same day that correspondence is placed for collection and mailing, it is deposited in the ordinary course of business with the United States Postal Service in a sealed envelope with postage fully prepaid.
5. The envelope was addressed and mailed as follows:
   a. **Name** of person served: Eric M. Katz, Supervising Deputy Attorney General
   b. **Address** of person served:
      300 South Spring Street, Suite 1702
      Los Angeles, CA 90013

   ☐ The name and address of each person to whom I mailed the documents is listed in the *Attachment to Proof of Service by First-Class Mail—Civil (Persons Served)* (POS-030(P)).

I declare under penalty of perjury under the laws of the State of California that the foregoing is true and correct.

Date: September 11, 2015

Margaret C. Wilson
(TYPE OR PRINT NAME OF PERSON COMPLETING THIS FORM)

▶ Margaret C Wilson
(SIGNATURE OF PERSON COMPLETING THIS FORM)

EXHIBIT 8

KAMALA D. HARRIS
Attorney General of California
ERIC M. KATZ
Supervising Deputy Attorney General
State Bar No. 204011
CAROL A. Z. BOYD
Deputy Attorney General
State Bar No. 165988
300 South Spring Street, Suite 1702
Los Angeles, CA 90013
Telephone: (213) 897-2630
Fax: (213) 897-2802
E-mail: Eric.Katz@doj.ca.gov
E-mail: Carol.Boyd@doj.ca.gov
Attorneys for Respondent
State Water Resources Control Board

[EXEMPT FROM FILING FEES – GOV. CODE § 6103]

SUPERIOR COURT OF THE STATE OF CALIFORNIA

COUNTY OF LOS ANGELES

CENTRAL BRANCH

ANDREW C. WILSON,

Petitioner,

v.

STATE WATER RESOURCES CONTROL BOARD,

Respondent.

Case No. BS149632

RESPONDENT'S OPPOSITION TO PETITIONER'S MOTION FOR NEW TRIAL

[Declaration of Shahla D. Farahnak in Support of Opposition to Motion for New Trial, filed herewith]

Action Filed: July 3, 2014
Trial Date: July 28, 2015

Hearing Date: October 15, 2015
Hearing Time: 9:30 a.m.

Dept: 85
Judge: Hon. James C. Chalfant

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Page

CASES

TABLE OF AUTHORITIES
(continued)

Page

STATUTES

REGULATIONS

OTHER AUTHORITIES

INTRODUCTION

Petitioner Andrew C. Wilson's (Petitioner) motion for new trial should be denied because the court did not err during trial, but even if it did, none of the three alleged errors Petitioner claimed occurred could have affected the writ hearing's outcome. First, Petitioner's claim that the State Water Resources Control Board (Board) had a mandatory duty to consult with the California Department of Public Health (CDPH) prior to adopting the general waste discharge requirements for Recycled Water Use (General WDRs) and failed to do so, should be rejected. The Board in fact did consult with CDPH, despite not having an mandatory duty to do so. Second, Petitioner's attack on the court's presumption that CDPH's regulation addressed all potential human health risks from the use of recycled water in orchards is itself an unsupported argument. Third, Petitioner's rehash of whether or not *Topanga Association for a Scenic Community v. County of Los Angeles* (1974) 11 Cal.3d 506 (*Topanga*) applies is irrelevant, because the court found that even if *Topanga* applies, the Board satisfied its requirements. Petitioner's motion for new trial should be denied.

ARGUMENT

I. PETITIONER'S NEW "DUTY TO CONSULT" ARGUMENT SHOULD BE REJECTED AS BOTH UNTIMELY AND WRONG.

A. Petitioner Did Not Argue at Trial That the Board's General WDRs Are Defective Because It Breached a Purported Duty to Consult with CDPH.

Petitioner argues that the Board had a mandatory duty to consult with CDPH prior to adopting the General WDRs, and that the Board failed to discharge that mandatory duty. To his credit, Petitioner expressly concedes that his "duty to consult" argument is an entirely new argument that he did not raise at trial. (Petitioner's Memorandum of Points and Authorities in Support of Motion for New Trial (Motion) at p. 5:1-2.) The Board recognizes that under certain circumstances a petitioner can raise a new legal theory not argued at trial if it is based on undisputed facts. (*Hoffman-Haag v. Transamerica Ins. Co.* (1991) 1 Cal.App.4th 10, 15-16.) While Petitioner frames the argument as a new theory as to why there is a lack of substantial evidence to support the Board's findings, in reality Petitioner is seeking a different type of writ

under Code of Civil Procedure section 1085, to order the Board to consult with CDPH prior to adopting the General WDRs. (Motion, Exhibit A [Proposed Judgment], p. 2 ¶ 3.) To support that argument, Petitioner would need to amend his pleadings, post-trial, in order to state a new cause of action, as Petitioner has never previously alleged that the Board had a duty to consult with CDPH, and never alleged that he was seeking a writ of mandate to enforce an alleged mandatory duty under Code of Civil Procedure section 1085. (*US Ecology, Inc. v. State of California* (2001) 92 Cal.App.4th 113, 138 [discussing section 1085 writs].) This strategy should be rejected at this late date.

B. The Board Consulted With CDPH Before Adopting the General WDRs.

Petitioner's argument should be rejected as factually flawed because the Board, in fact, did consult with CDPH prior to adopting the General WDRs. (See Declaration of Shahla D. Farahnak in Support of Opposition to Motion for New Trial (Farahnak Decl.), filed herewith.) Because the Petitioner's petition and briefing did not assert a duty to consult, the Board overlooked certain documents related to its consultation with CDPH that otherwise belonged in the administrative record. Should the court grant a new trial, the Board would supplement the administrative record with the exhibits attached to the Farahnak Declaration, filed herewith, to demonstrate that it did consult with CDPH, fatally undermining Petitioner's new argument.

As set forth in the exhibits to the Farahnak declaration, Board staff met and conferred with CDPH staff prior to the Board's June 4, 2014 adoption of the General WDRs, received CDPH input, and revised the General WDRs in response to that input. On April 8, 2014, the Board (including Board member Tam Doduc and Executive Officer Tom Howard) met with a CDPH representative to discuss the draft General WDRs. (*Id.* Ex. A.) The Board set up a second meeting on May 15, 2014 with CDPH. (*Id.* Exs. B and D.) Prior to that meeting, CDPH staff member Brian Bernados submitted six pages of comments on the draft General WDRs. (*Id.* Ex. C.) Many of CDPH's comments were incorporated into the draft General WDRs, as reflected in a redline version of the General WDRs. (*Id.* Ex. E.) None of CDPH's edits suggested additional monitoring requirements for irrigated agriculture. (*Id.* ¶ 6.) Finally, Brian Bernados from CDPH

was present at the Board's June 3, 2015 public adoption hearing and commented favorably on the General WDRs. (AR 210-214.)

The foregoing demonstrates that even if Petitioner is correct that the Board had a duty to consult with CDPH (it does not) and Petitioner is empowered to enforce that duty (he does not), the Board satisfied that duty.

C. The Board Did Not Have a Mandatory Duty to Consult With CDPH Prior to Adopting the General WDRs.

Even if the Board had not consulted with CDPH, that fact would not warrant granting a new trial because the Board did not have a mandatory duty to do so in this circumstance. Petitioner's new theory is that the Board had a mandatory duty to consult with CDPH before adopting the General WDRs. (Motion at pp. 4-6.) If pursued, the claim would have to be based on Code of Civil Procedure section 1085 (Section 1085). As the court is well aware, Section 1085 authorizes the issuance of a writ of mandate "to compel the performance of an act which the law specifically enjoins, as a duty resulting from an office, trust, or station, ..." (Code Civ. Proc., § 1085.) Two basic requirements are essential to the issuance of a writ: (1) a clear, present and usually ministerial duty on the part of the respondent agency, and (2) a clear, present and beneficial right in the petitioners to the performance of that duty. (*Loder v. Municipal Court (San Diego)* (1976) 17 Cal.3d 859, 863; see also *State of California v. Superior Court* (1974) 12 Cal.3d 237, 247; *Sierra Club v. Cal. Dept. of Parks and Recreation* (2012) 202 Cal.App.4th 735, 740 ["It is settled that traditional mandamus only lies to compel the performance of a clear, present ministerial duty"].) A ministerial duty is one that an agency is required to perform in a prescribed manner without any exercise of judgment or discretion. (*US Ecology, Inc. v. State of California, supra,* 92 Cal.App.4th at p. 138.) A person seeking the issuance of a writ of mandate bears the burden of pleading and proving the facts on which its claim is based. (*Polster v. Sacramento County Office of Educ.* (2009) 180 Cal.App.4th 469, 670.) However, Petitioner did not allege this claim in his petition, did not brief the claim, and did not argue it at trial.

1. The Board does not have an implicit mandatory duty to consult with CDPH.

Petitioner does not point to any specific statute or regulation for the proposition that the Board has a mandatory duty to consult with CDPH prior to adopting General WDRs. Rather, Petitioner locates this alleged mandatory duty to consult as being "implicit in the statutory scheme." (Motion at p. 5:8.) Petitioner's claimed "implicit" duty is not enforceable under section 1085 because it would fail the "clear and present" prong. (E.g., *US Ecology, Inc. v. State of California, supra,* 92 Cal.App.4th at pp. 137-139.) Courts cannot impose a duty on a public agency to take an action absent a clear and present legal obligation to do so, even if the court were to find that "common sense" suggests that the agency should do so. (*Hutchinson v. City of Sacramento* (1993) 17 Cal.App.4th 791, 798.) "Mandate will not issue to compel action unless it is shown the duty to do the thing asked for is plain and *unmixed with discretionary power or the exercise of judgment.*" (*County of San Diego v. State of California* (2008) 164 Cal.App.4th 580, 596, italics in original.) Because Petitioner cannot identify a "clear and present" source for the alleged duty, the argument fails.

a. Water Code sections 13523 and 13523.1 do not create a mandatory duty on the Board to consult with CDPH before adopting General WDRs.

The Board adopted the General WDRs pursuant to its authority under Water Code sections 13263, subdivision (i). (AR 9.) Nothing in this Water Code section suggests that the Board had an obligation to consult with CDPH prior to adopting the General WDRs.

Without explanation, Petitioner cites two different Water Code provisions – sections 13523 and 13523.1 – as allegedly implied sources of the Board's purported duty to consult with CDPH. (Motion at p. 5:24-25.) Neither of these statutes imposes such a duty on the Board when adopting General WDRs because those sections deal with separate administrative processes that the Board did not undertake here. Water Code section 13523 deals with a regional water board's issuance of an individual "water reclamation requirement" to a water recycling facility and Water Code section 13523.1 deals with a regional water board's issuance of "master reclamation permits." (Wat. Code, §§ 13523, 13523.1.) General WDRs issued by the Board are neither "water

reclamation requirements" nor "master reclamation permits," and so on their face the statutes do not apply. Petitioner's citation to these sections actually underscores the point that while the regional boards may have a duty to consult with CDPH when adopting water reclamation requirements or a master reclamation permit, there is no corresponding duty on the Board to so when adopting General WDRs. (Cf. Wat. Code, §§ 13263, 13523, 13523.1.)

b. The 1997 Memorandum of Agreement does not create a mandatory duty on the Board to consult with CDPH before adopting General WDRs.

Petitioner also claims that the 1997 Memorandum of Agreement (MOA) between the Board and CDPH is an implied source for the Board's alleged mandatory "duty to consult." Yet the MOA does not impose a mandatory duty on the Board to consult with CDPH before the Board adopts General WDRs, especially where, as here, the Board's action is taken in total compliance and reliance on CDPH's prior regulation. (Petitioner's Request for Judicial Notice [RJN], Ex. D.) The Board's decision to accept and rely on CDPH's prior regulatory decision – irrigating orchards with recycled water is safe for human health – fulfills the MOA's express purpose "to assure the respective authority of [CDPH and the Board] relative to the use of [recycled] water will be exercised in a coordinated and cohesive manner designated to eliminate overlap of activities, duplication of effort, gaps in regulation, and inconsistency of action." (Petitioner's RJN, Ex. D, p. 1 [Section I, second paragraph].) Section IV of the MOA lists the parties' "program provisions and commitments" and none of them involve the Board consulting with CDPH with respect to the Board's adoption of General WDRs. (*Id.* at pp. 6-7 [but see Section I.A, *supra*, describing how the Board in fact consulted with CDPH].)

2. Any duty to consult that the Board had is not enforceable by Petitioner.

Even if the Water Code or the MOA created a duty to consult, that duty can only be enforced by Petitioner if there there is "a clear, present and beneficial right *in the petitioners* to the performance of that duty." (*Loder v. Municipal Court (San Diego), supra,* 17 Cal.3d at p. 863, italics added.) There is no indication in Water Code sections 13520 or 13521.1 were intended to be privately enforced. Nor is there any indication in the MOA that either the Board or CDPH

intended that it benefit and be enforceable by third parties. In order to seek to enforce its terms, Petitioner has the burden to show that when the Board and CDPH entered into the MOA they "intended to benefit the unnamed party [petitioner] and the agreement reflects that intent." (*Sessions Payroll Management, Inc. v. Noble Const.* (2000) 84 Cal.App.4th 671, 680-681; *Spinks v. Equity Residential Briarwood Apartments* (2009) 171 Cal.App.4th 1004, 1022-1023.) Petitioner has made no such showing, and review of the MOA itself reveals no such intent. (Petitioner's RJN, Ex. D.)

II. PETITIONER'S CHALLENGE TO THE COURT'S CITATION TO EVIDENCE CODE SECTION 664 SHOULD BE REJECTED.

Petitioner claims that there was not evidentiary support for the court's presumption that CDPH's regulation – California Code of Regulations, title 22, section 60304 (section 60304), which provides that it safe from human health standpoint to irrigate orchards with recycled water – was adopted to be protective of human health from all potential risks to human health, including perchlorate in orchards. Petitioner now speculates that CDPH might have decided to ignore "systemic" risks when it adopted section 60304 because those risks, according to him, differ between orchards planted in the desert and those on the coast, and therefore those risks were not amenable to statewide regulation. (Motion at pp. 9-11.) Petitioner's claim is inconsistent with the plain terms of CDPH's legislative mandate, and Petitioner's new theory is wholly unsupported by the administrative record.[1]

There is no textual support in Water Code sections 13520 or 13521 to support Petitioner's novel new proposed argument that the Legislature tasked CDPH with adopting recycling criteria that were not protective against all types of human health risk, whether "systemic" or not, in all parts of the state. The statute expressly provides that CDPH is to adopt "*uniform statewide* recycling criteria for each varying type of use of recycled water where the use involves the

[1] The court should not grant Petitioner a new trial to advance this argument, as it would be barred by the administrative exhaustion doctrine. (*Abelleira v. District Court of Appeal, Third District* (1941) 17 Cal.2d 280, 292; *Hagopian v. State* (2014) 223 Cal.App.4th 349, 371.) Nowhere in the administrative record, and certainly not in Petitioner's comments to the Board, did Petitioner argue that section 60304 was not adopted to protect human health statewide and allow for the safe irrigation of orchards in all of California's climate types, including the desert.

protection of human health." (Wat. Code, § 13521, italics added.) Petitioner presented no evidence at trial or in his Motion to support his argument that the Legislature directed CDPH to ignore allegedly "systemic risks," or that CDPH ignored those risks, whether or not directed by the Legislature to do so. The only reasonable interpretation of its statutory mandate was that CDPH was tasked with adopting recycling criteria that can apply uniformly statewide – both in the desert and elsewhere – and protect against any human health risk posed by the use of recycled water, whether systemic or otherwise. There is absolutely no indication that CDPH did not comply with its legislative mandate to adopt uniform statewide recycling criteria when it adopted section 60304 and concluded that orchards – statewide – can safely be irrigated with recycled water. The court did not err in presuming that CDPH fulfilled its statutory mandate to adopt "uniform statewide recycling criteria" when it adopted section 60304. As the court found, such a regulation, adopted for the statutory purpose of protecting human health, is substantial evidence in support of the Board's finding that the General WDRs are protective of human health. (Petitioner's RJN, Ex. B at p. 9 ["The regulations of an agency with appropriate expertise can provide substantial evidence in support of another agency's decision. Oakland Heritage Alliance v. City of Oakland ('Oakland') (2011) 195 Cal.App.4th 884, 903-04"].)

Petitioner claims that there was no evidence at trial that CDPH considered systemic risks when it adopted section 60304. Petitioner misunderstands that it was his burden to present to the Board and then to the court, sufficient evidence to diminish the persuasiveness of the evidence that Board relied on to make its finding to a level of insubstantiality. Petitioner's post-trial speculation as to what CDPH may or may not have done when it adopted section 60304 is not sufficient to make what is otherwise substantial evidence – section 60304 – into evidence of a less than substantial nature in support of the Board's finding.

The legal question in this case remains whether, based on all the evidence presented to the Board including Petitioner's comments, a reasonable person could not have found General WDRs were safe for human health, as the Board found. (*Hagopian v. State* (2014) 223 Cal.App.4th 349, 360; AR 6-7 ¶¶ 9-10.) The court has to presume that substantial evidence supports the Board's decision, and resolves all reasonably doubts in favor of the Board's decision. (*Ibid.*) Petitioner's

post-trial speculation that CDPH may not have considered "systemic risk" as he now defines it, and the difference between the risk to human health posed by irrigating orchards with recycled water in the desert as compared to the coast, is not sufficient to undermine the evidence the Board relied on. Petitioner's Evidence 664 argument is not a sufficient basis on which the court can or should grant a new trial.

III. PETITIONER'S ARGUMENT REGARDING WHETHER ADOPTION OF GENERAL WDRS IS QUASI-LEGISLATIVE OR ADJUDICATIVE IS IMMATERIAL.

The court should reject Petitioner's claim that he is entitled to a new trial because the court made a legal error in concluding that *Topanga* did not apply to the Board's adoption of General WDRs. The court did not err, but even if it did, Petitioner suffered no prejudice because the court found that the Board's findings satisfied *Topanga*.

A new trial can be granted only if the claimed error, if reversed, would likely change the outcome of the trial. (Cal. Const. Art. VI, § 13; *Bristow v. Ferguson* (1981) 121 Cal.App.3d 823, 826 ["If it clearly appears that the error could not have affected the result of the trial, the court is bound to deny the motion"].) Petitioner cannot make that showing here because the court's conclusion that *Topanga* did not apply is not essential to the court's ultimate conclusion to uphold the General WDRs. That is because the court also found that even if *Topanga* applied, the Board complied with it. (Petitioner's RJN, Ex. A, at pp. 8-10.) Thus, even if the court was inclined to revisit its conclusion that *Topanga* did not apply to the Board's adoption of General WDRs, conducting a new trial would be an idle act because the court already found that the Board fully complied with *Topanga*. The error, if any, was not prejudicial because it would not change the trial's outcome. (Cal. Const. Art. VI, § 13; Civ. Proc. Code, § 475.)

Furthermore, nothing in Petitioner's Motion takes away from the force of the court's conclusion with respect to the adoption of General WDRs having, at most, mixed quasi-legislative and adjudicative characteristics. (Petitioner's RJN, Ex. A, at p. 8.) The court's conclusion as to whether or not adoption of the General WDRs is quasi-legislative or adjudicative does not bind the Board in future administrative proceedings to conduct such future proceedings as either quasi-legislative or adjudicatory.

CONCLUSION

For all of the foregoing reasons, the State Water Board respectfully requests that the court deny Petitioner's motion for new trial.

Dated: September 28, 2015

Respectfully Submitted,

KAMALA D. HARRIS
Attorney General of California

ERIC M. KATZ
Supervising Deputy Attorney General
Attorneys for Respondent
State Water Resources Control Board

LA2014512195
51919200.doc

DECLARATION OF SERVICE BY U.S. MAIL

Case Name: **Andrew C. Wilson v. State Water Resources Control Board**

Case No.: **BS149632**

I declare:

I am employed in the Office of the Attorney General, which is the office of a member of the California State Bar, at which member's direction this service is made. I am 18 years of age or older and not a party to this matter; my business address is 300 South Spring Street, Suite 1702, Los Angeles, CA 90013.

On September 28, 2015, I served the attached **RESPONDENT'S OPPOSITION TO PETITIONER'S MOTION FOR NEW TRIAL** by placing a true copy thereof enclosed in a sealed envelope with postage thereon fully prepaid, in the United States Mail at Los Angeles, California, addressed as follows:

Mr. Andrew C. Wilson
7468 Dufferin Ave.
Riverside, California 92504
In Pro Se

I declare under penalty of perjury under the laws of the State of California the foregoing is true and correct and that this declaration was executed on September 28, 2015, at Los Angeles, California.

Beatriz Davalos
Declarant

Beatriz Davalos
Signature

LA2014512195
51680257.doc

EXHIBIT 9

ANDREW C. WILSON
State Bar No. 133062
7468 Dufferin Avenue
Riverside, CA 92504
Telephone: (951) 687-4471
E-mail: acwilson11@yahoo.com

Petitioner In Pro Se

SUPERIOR COURT OF THE STATE OF CALIFORNIA

COUNTY OF LOS ANGELES

CENTRAL DISTRICT

ANDREW C. WILSON, Petitioner, **v.** **STATE WATER RESOURCES CONTROL BOARD,** Respondent.	Case No. BS149632 **PETITIONER'S REPLY BRIEF IN SUPPORT OF MOTION FOR NEW TRIAL** Dept: 85 Judge: The Honorable James C. Chalfant Hearing Date: October 15, 2015 9:30 a.m. Trial Date: July 28, 2015 Action Filed: July 3, 2014

TABLE OF CONTENTS

TABLE OF AUTHORITIES

I. INTRODUCTION

One of the key purposes of the public comments and hearing on the general permit was to determine if recycled water is safe enough for broad use under a statewide general permit as opposed to individual permits. The high evidentiary burden adopted by the Court, together with the unreasonable position that the Board is taking in response to this new trial motion, make public participation in the process meaningless and completely defeat the goal of protecting the public health.

All the issues that Petitioner has raised are properly raised on this motion for new trial. Petitioner's proposed judgment is a simple solution to this case.

II. ARGUMENT

A. The Record Contains No Evidence Showing That the Board Fulfilled Its Duty To Consult With and Receive in Evidence the Views of CDPH on the Public Health Issue Raised by Petitioner's Comment.

(1) The Memorandum of Agreement Requires That the Board's Decisionmakers Not Take A Public Health Position Contrary To the Position of CDPH When Issuing General Permits.

At trial counsel for the Board stated that the Memorandum of Agreement (MOA) requires the Board's decisionmakers, when issuing general permits, not to take a position on public health that is contrary to the position of CDPH. (RT 20; Petitioner's Request For Judicial Notice, Ex. D.) For that requirement to make sense, the Board's decisionmakers need to know what CDPH's position is on issues of public health that the Board will decide at general permit hearings. Further, that information must be received as evidence because the decisionmakers must base their decision only on the evidence presented at the hearing.

The Court has ruled that the Board may rely solely on Title 22 as evidence unless Petitioner meets an extremely high evidentiary burden. Petitioner interprets the Court's ruling to mean that if Petitioner meets the high evidentiary burden, then, under the substantial evidence rule, the Board may not merely rely on Title 22, but must consult with and receive in evidence CDPH's views on Petitioner's comment.

Petitioner contends on this new trial motion that the Court has set the evidentiary burden too high. The correct evidentiary burden needs to be lower and in harmony with the requirement

that the decisionmakers must not take a public health position contrary to CDPH. Petitioner has met the correct burden, and the Board's mere reliance on Title 22, and failure to receive in evidence CDPH's views, violates the substantial evidence rule.

In response, the Board argues that when issuing general permits, the Board is *never* under a duty to consult with and receive the views of CDPH in evidence. Under that legal theory, even if Petitioner meets the Court's high evidentiary burden, the Board may rely merely on Title 22. The Court should reject this insupportable theory as contrary to the Court's ruling.

At the hearing in the instant case, the Board brought up the MOA when the Court inquired about the Governor's Order, which provided: "The Water Board will adopt statewide general waste discharge requirements to facilitate the use of treated wastewater that meets standards set by the Department of Public Health, in order to reduce demand on potable water supplies." (AR 397, RT 19-22.) (At the hearing the parties mistakenly referred to the MOA as the "MOU.")

> THE COURT: Well, what if Mr. Wilson had shown that perchlorate is a new problem, nobody knew about it until two years ago? It's a serious public health problem. And he also showed that the Department of Public Health did not consider perchlorate when it issued its regulations. Would the Water Board have been bound by the Governor's direction, or could it consider perchlorate issues?
>
> MR. KATZ: Well, that's interesting. It would be inconsistent with the MOU to act - -
>
> THE COURT: Is the MOU between the two agencies?
>
> MR. KATZ: Correct. *It would be inconsistent with the MOU for the State Board to take a contrary public health position to the Department of Public Health, and I believe there is a dispute resolution process in the MOU if there is such a disagreement.*
>
> THE COURT: *Well, arguably, by my hypothetical, there wouldn't be any disagreement.* The Department of Public Health would have never considered perchlorate. RT 19-20 (italics added).

In the above passage, the Board's counsel expressly states that the MOA applies to the Board's decision making *when issuing general permits*. The Court's response indicates that the

Court agrees the Board should not take a position contrary to CDPH. On this motion for new trial, Petitioner seeks to clarify the effect of the MOA under the Court's ruling.

Properly interpreted, the MOA allows the Board to require additional precautionary measures if the CDPH believes, in light of Petitioner's evidence, that a reasonable person would undertake additional safety precautions beyond title 22 requirements. In that situation, a decision by the Board to include those additional precautions in the permit would be entirely consistent with the position of the CDPH, and therefore proper under the MOA. Moreover, the Board's refusal to include the additional requirements would violate the MOA. In this respect, the MOA protects the public because it prevents the Board from adopting permits that the CDPH believes would threaten public health. However, under the Court's hypothetical, compliance with the MOA cannot be determined without evidence of the current position of the CDPH on exactly what additional safety precautions are appropriate.

Under the Court's hypothetical, the title 22 regulations alone are not substantial evidence supporting the Board's finding that the general permit protects public health. More evidence is required. The Court answered its own hypothetical and stated that the Board could not just rely on Title 22:

> MR. WILSON: . . . . I mean, I just - - I'm trying to - - I do think that your question about the Memorandum of understanding, you know, could they have stepped in and required more stringent requirements in Title 22, and then Mr. Katz suggested, well, that might have violated their Memorandum of Understanding, but I think it's really a question of their statutory authority. Because their statutory authority allowed them to do it, and if they bargained that way with some contract, I'm not sure he's saying that.
>
> THE COURT: I fully understand. And under my hypothetical *I would expect them to do something. I would not expect them to say, "Well, we'll just rely on their regulation," would not expect them to do that.* Whether their Memorandum of Understanding - -
>
> MR. WILSON: Whatever it says.
>
> THE COURT: Yes.

MR. WILSON: Right.

THE COURT: But that's not what we have here, and I do think they get to rely on it. Remember, we're talking only about substantial evidence, not whether an independent review of the evidence says there might be a problem here, which is what your evidence presents, but, rather, is there substantial evidence, whatever, however good your evidence is, is there substantial evidence for the Board to - - for the Water Board to rely on, and there is. RT 21-22 (italics added).

In the Court's hypothetical, Petitioner would be able to overcome the presumption that the CDPH did their job. Overcoming the presumption means additional evidence is necessary to satisfy the substantial evidence rule. The Court stated, "I would expect them to do something." (*Id.*) To comply with the MOA, that additional evidence must be evidence of the views of the CDPH on Petitioner's comment.

(2) The Issues Raised By Petitioner Must Be Adjudicated In the Pending Cause of Action.

According to the Board, not only is it never under a duty to consult with CDPH when issuing general permits, the issue of whether that duty exists cannot be adjudicated in this cause of action for administrative mandamus, but must be litigated in a new cause of action for ordinary mandamus to enforce a ministerial duty under Code of Civil Procedure section 1085.

Under the Court's ruling, as interpreted by Petitioner, if Petitioner had met the high burden, the Board could not have relied solely on title 22, but would have had a duty to consult with and receive the views of CDPH in evidence. If there is never a duty, the Board could always rely solely on title 22, and the Court's ruling is wrong. Questions of the whether the Court's ruling is wrong must be litigated in this cause of action. The Legislature has provided the writ of administrative mandamus as the sole remedy to set aside a general permit. (Water Code §§ 13330 (a), (e), (g).) As the sole remedy it is necessarily an adequate remedy, and allows for litigation of all issues necessary to fully settle any controversy over the validity of the agency's action.

Moreover, Petitioner is not seeking to compel agency action, he is seeking to set aside agency action. Petitioner's Proposed Judgment does not set aside agency action. It is intended to

be a good faith practical solution to end this case that would be entered only with the Board's consent. It is not in proper format for a contested judgment in this case. If a new trial is granted, and there is no amicable resolution, then, as stated in Petitioner's petition, Petitioner seeks a judgment setting aside the general permit.

As stated in the Court's decision, a petitioner has the burden to show that the agency failed to regularly perform its duty. (Decision, p. 2) For example, the petitioners in *Topanga* would have shown that the zoning agency failed to perform its duty if they had demonstrated that the agency had not received substantial evidence showing that the character of the neighboring property was different from the land in question. (*Topanga Assn. for a Scenic Community v. County of Los Angeles* (1974) 11 Cal.3d 506, 519 n. 19.) If the substantial evidence rule requires that certain evidence be received, the agency has a "duty" to receive that evidence.

In this case, the purpose of consulting with CDPH is not for the sake of consulting, it is to receive CDPH's views in evidence. If Petitioner meets the appropriate evidentiary burden, the Board has a duty to receive in evidence CDPH's views on Petitioner's comment.

The duty to receive CDPH's views in evidence, like the duty to receive evidence in *Topanga*, is not a ministerial duty.

Traditionally, ordinary mandamus has been used to obtain two different forms of relief: (1) to *compel* agency action that is ministerial, or (2) to *set aside* agency action for abuse of discretion. Different conditions must be established for the different forms of relief. Agency rulemaking may be set aside for abuse of discretion. Abuse of discretion includes rules that are "arbitrary, capricious, or lacking in evidentiary support." (*California Hotel & Motel Assn. v. Industrial Welfare Com.* (1979) 25 Cal.3d 200, 212.) Legislatures have the power to require agencies they create to consult with and receive the recommendations of other agencies when they are engaged in rulemaking. If the agency fails to consult and receive the recommendations, the rule may be set aside for abuse of discretion due to lack of evidentiary support. The rules associated with compelling ministerial action do not apply.

Similarly, legislatures have the power to require agencies they create to consult with and receive the recommendations of other agencies when they are engaging in mixed

rulemaking/adjudication or engaging in pure adjudication. If the agency fails to comply, the agency's action may be set aside for "abuse of discretion" due to lack of evidentiary support. (Code of Civil Procedure sections 1094.5 (b) and (c) define "abuse of discretion" to include lack of substantial evidence.) As is the case when setting aside a rule, the special rules associated with compelling ministerial action do not apply.

The cases on section 1085 cited by the Board explain the special rules that apply only to compelling ministerial action. Those rules are not applicable here.

(3) Petitioner's Arguments Are All Timely.

All of Petitioner's arguments are timely because Petitioner may raise new legal theories for the first time on a motion for new trial. All of Petitioner's arguments are responsive to theories adopted by the Court that Petitioner was unaware of until trial.

(4) The Board's New Documents Do Not Show That the Board's Decisionmakers Received in Evidence the Views of CDPH on Petitioner's Comment and the New Documents Do Not Belong in the Administrative Record.

The Board offers five new documents that it claims "fatally" undermine "Petitioner's new argument." (Respondent's Brief, at p. 2.) However, none of the documents are relevant because none show that the Board consulted with CDPH about Petitioner's comment or that the Board's decisionmakers received in evidence the views of CDPH on his comment. These documents are properly excluded from the administrative record and Petitioner objects to the Court receiving any of them in evidence or taking judicial notice of them.

Four of the five documents concern CDPH comments that were generated on or before May 15, 2014. (Declaration of Shahla Farahnak, Exs. A, B, C, and D.) Petitioner submitted his comment to the Board on May 27, 2014. It is impossible for these CDPH comments to have addressed Petitioner's comment because these CDPH comments were all generated before Petitioner submitted his comment to the Board. The fifth document purports to be an undated draft of the general permit that incorporates many (but apparently not all) of those CDPH comments. (Declaration of Shahla Farahnak, Ex. E; Respondent's Brief, p. 2.) Nothing in the draft permit shows that CDPH received Petitioner's comment or that the Board consulted with

CDPH about Petitioner's comment.

The Board also argues that Mr. Bernardos of CDPH "commented favorably" on the general permit at the hearing (AR 210-214). (Respondent's Brief, pp. 2-3.) Nothing in the transcript shows that Mr. Bernardos received or reviewed Petitioner's comment or that he communicated his views on Petitioner's comment to the Board's decisionmakers.

The Board contends that the five new documents "belonged in the administrative record." (Respondent's Brief, p. 2.) However, as far as Petitioner is aware, none of these documents have ever been previously publicly disclosed. During the administrative proceeding the Board's staff never disclosed that they were offering any of these documents in evidence, and the decisionmakers never disclosed that they decided to receive any of them in evidence. These documents are properly excluded from the administrative record

(5) The Board Wrongly Refuses To Acknowledge Any Duty To Consult With CDPH.

The over-arching purpose of the MOA is "to assure the respective authority of [CDPH and the Board] relative to the use of [recycled] water will be exercised in a coordinated and cohesive manner designed to eliminate overlap of activities, duplication of effort, gaps in regulation, and *inconsistency of action*." (MOA, p. 1, italics added.) The over-arching purpose of the MOA must be consistent with the overarching purpose of the statutory scheme. Statutes must be interpreted to effectuate their purpose, and not defeat it.

Petitioner in his brief in support of the motion for new trial explained that the evidentiary burden imposed by the Court is unreasonably high. As a factual matter, an ordinary person could not be expected to include with his comments evidence of what CDPH had before it when it adopted Title 22. The resulting failure to meet the burden would require the rejection of virtually all public comments advocating protective measures beyond title 22, regardless of the actual validity of the comments. The Board in its brief did not dispute Petitioner's contentions.

Due to a failure to meet the high burden, under the Court's ruling the Board may take a public health position in conflict with CDPH. The burden proposed by Petitioner is the natural and obvious burden that prevents inconsistency of action. Public health issues may be divided

into two groups: those issues where it is possible for reasonable minds to differ, and those where it is not possible for reasonable minds to differ. There is no reason for inter-agency consultation on the latter group. There is, however, sound reason for consultation on issues in the former.

If, based on the evidence, it is possible for reasonable minds to differ, then by definition, it is possible that the position of the Board may differ from the position of CDPH.

To ensure maximum protection of public health, the potential for conflict must be eliminated. It is, in fact, easily eliminated by simply receiving evidence of the CDPH's views on the comment in question.

The Board asserts that while regional boards "may have a duty to consult with CDPH," it has no such duty. (Respondent's Brief, p. 5.) The Board argues that Water Code section 13263, which authorizes the general permit in this case, contains no specific language providing for consultation, as does Water Code section 13523, which authorizes regional boards to issue individual permits. However, under the Court's ruling, if the high burden is met, then the Board has a duty to consult and receive in evidence the views of CDPH.

It is important to note that the Board reads into section 13263 words that are not there – words that grant authority to issue a general permit under which individual users of recycled water may apply to be covered. Based on that implied authority, the general permit provides that individual users of recycled water covered under existing individual water recycling requirements may "apply for coverage under this General Order." (AR 15.) The implication of that authority brings with it the concomitant duty to consult.

B. <u>The Court's Application of Evidence Code § 664 Assumes Facts Not In Evidence</u>.

The Board's attorneys contend that the "*only* reasonable interpretation" of the enabling statute is that CDPH had a duty to include protection against systemic risk. (Respondent's Brief, p. 7.) That contention is not supported by the statutory language or any other evidence in the record. The Board's attorneys also argue:

> "Petitioner claims that there was no evidence at trial that CDPH considered systemic risks when it adopted section 60304. Petitioner misunderstands that it was *his burden* to present to the Board and then to the court, sufficient evidence to diminish the persuasiveness of the *evidence that the Board relied on* to make *its finding* to a level of insubstantiality. Petitioner's post-trial speculation as to what CDPH may or may not have

done when it adopted section 60304 is not sufficient to make what is otherwise substantial evidence – section 60304 – into evidence of a less than substantial nature in support of the *Board's finding*." (Respondent's Brief, at p. 7, italics added.)

In the above passage the "Board's finding" cannot refer to a finding that the alleged duty exists. The decisionmakers never invoked the presumption and never made a finding that the alleged duty exists. The "evidence that the Board relied on" cannot refer to evidence supporting a finding that the duty exists. The existence of the alleged duty is an essential fact underlying the presumption of Evidence Code section 664. Findings on essential facts cannot be implied in the reviewing court. There must be findings on all facts which as a matter of law "'are essential to sustain [the decision].'" (*Environmental Protection Info. Ctr. v. Cal. Dept. of Forestry and Fire Protection* (2008) 44 Cal.4th 459, 517, quoting *Sierra Club v. Cal. Coastal Com.* (1993) 19 Cal.App.4th547, 556.)

Not only is there no evidence supporting the interpretation advocated by the Board's attorneys, but Petitioner also introduced evidence that detracts from that interpretation. Petitioner submitted evidence that systemic risk is highly variable because it depends on climate and crop species. (AR 622) Systemic risk is lower near the coast than in the deserts because the water uptake and demand is higher in the hotter desert climate. (Id.) Enforcing statewide a strict safety level appropriate only for the desert would tend to defeat the state's goals of increased recycle water use. The enabling statute is properly interpreted to give CDPH the discretion to decide whether or not to include systemic risk in statewide criteria.

No evidence was introduced by Board staff or anyone else that contradicted the evidence submitted by Petitioner. Nevertheless, it appears that the Board's attorneys have either rejected or chosen not to believe Petitioner's evidence, as the Board's brief states that Petitioner's position "is wholly unsupported by the administrative record." (Respondent's Brief, at p. 8.) It is not clear that the Board's decisionmakers would evaluate Petitioner's evidence in the same way, and opinions of attorneys are not a valid substitute.

Neither the Board's attorneys not the Court may invade the province of the Board and find the existence of the alleged duty and invoke the presumption. The decisionmakers never invoked the presumption.

The Board also argues that the administrative exhaustion doctrine bars Petitioner from challenging the existence of the alleged official duty. There is no bar because Petitioner first learned of the presumption and the alleged duty on the day of the trial.

C. The Court's Decision That Findings Are Not Required Conflicts With *Topanga*.

The Court has decided that findings are not required. The Board argues that the Court's decision does not "bind" the Board in "future administrative proceedings." (Respondent's Brief, at p. 8.) However, if the decision is not set aside, it will be res judicata and binding on Petitioner in future proceedings. If the Board in the future violates its duty to make findings, Petitioner will be estopped from challenging it under the doctrine of res judicata (or collateral estoppel). For this reason, setting aside the decision is not an "idle act."

Setting aside the decision will also likely change the outcome of the trial because it will prevent the Court from implying a finding by the decisionmakers that the alleged duty underlying the presumption of Evidence Code section 664 exists.

The Board presents no convincing argument in opposition to the principle that findings are required when there exists an independent legislative mandate to apply Code of Civil Procedure section 1094.5.

VIII. CONCLUSION

For all the foregoing reasons, Petitioner respectfully urges the Court to grant the relief requested at pp. 13-14 of Petitioner's Memorandum of Points and Authorities in Support of Motion For New Trial.

Dated: October 7, 2015

Respectfully submitted,

ANDREW C. WILSON

Andrew C. Wilson

Andrew C. Wilson
Petitioner In pro se

POS-030

ATTORNEY OR PARTY WITHOUT ATTORNEY (Name, State Bar number, and address):	FOR COURT USE ONLY
ANDREW C. WILSON STATE BAR NO. 133062 7468 Dufferin Avenue Riverside, CA 92504 TELEPHONE NO.: (951) 687-4471 FAX NO. (Optional): E-MAIL ADDRESS (Optional): acwilson11@yahoo.com ATTORNEY FOR (Name): In pro se	
SUPERIOR COURT OF CALIFORNIA, COUNTY OF Los Angeles STREET ADDRESS: 111 North Hill Street MAILING ADDRESS: CITY AND ZIP CODE: Los Angeles, CA 90012 BRANCH NAME: Central District - Stanley Mosk Courthouse	
PETITIONER/PLAINTIFF: ANDREW C. WILSON RESPONDENT/DEFENDANT: STATE WATER RESOURCES CONTROL BOARD	
PROOF OF SERVICE BY FIRST-CLASS MAIL—CIVIL	CASE NUMBER: BS149632

(Do not use this Proof of Service to show service of a Summons and Complaint.)

1. I am over 18 years of age and **not a party to this action.** I am a resident of or employed in the county where the mailing took place.

2. My residence or business address is:
   2806 Gertrude Street
   Riverside, CA 92506
3. On *(date)*: October 7, 2015 I mailed from *(city and state)*: Riverside, California
   the following **documents** *(specify)*:
   Petitioner's Reply in Support of Motion For New Trial

   ☐ The documents are listed in the *Attachment to Proof of Service by First-Class Mail—Civil (Documents Served)* (form POS-030(D)).
4. I served the documents by enclosing them in an envelope and *(check one)*:
   a. ☑ **depositing** the sealed envelope with the United States Postal Service with the postage fully prepaid.
   b. ☐ **placing** the envelope for collection and mailing following our ordinary business practices. I am readily familiar with this business's practice for collecting and processing correspondence for mailing. On the same day that correspondence is placed for collection and mailing, it is deposited in the ordinary course of business with the United States Postal Service in a sealed envelope with postage fully prepaid.
5. The envelope was addressed and mailed as follows:
   a. **Name** of person served: Eric M. Katz, Supervising Deputy Attorney General
   b. **Address** of person served:
      300 South Spring Street, Suite 1702
      Los Angeles, CA 90013

   ☐ The name and address of each person to whom I mailed the documents is listed in the *Attachment to Proof of Service by First-Class Mail—Civil (Persons Served)* (POS-030(P)).

I declare under penalty of perjury under the laws of the State of California that the foregoing is true and correct.

Date: October 7, 2015

Tom H. Wilson, Jr.
(TYPE OR PRINT NAME OF PERSON COMPLETING THIS FORM)

▶ Tom H. Wilson, Jr.
(SIGNATURE OF PERSON COMPLETING THIS FORM)

EXHIBIT 10

Andrew C. Wilson v. State Water Resources Control Board
BS 149632

Tentative decision on motion for new trial: denied

Petitioner Andrew C. Wilson ("Wilson") moves the court for a new trial on his petition for writ of mandate.

The court has read and considered the moving papers, opposition, and reply, and renders the following tentative decision.

A. Statement of the Case

1. Petition

Petitioner Wilson commenced this proceeding on July 3, 2014. The Petition alleges in pertinent part as follows. On June 3, 2014, the Board held a public meeting to receive evidence on the issue of whether grounds exist for prescribing general waste requirements for recycled water use. Wilson submitted written comments in opposition, which were received by the Board. These written comments discussed the danger of the chemical perchlorate, and included four scientific articles as exhibits.

The Board adopted a written order on June 3, 2104, entitled "General Waste Discharge Requirements for Recycled Water Use" (the "Order"). The Board decided in the Order that compliance with the California Department of Public Health recycling criteria, set forth in CCR title 22, is sufficient to protect against public health risks arising from the use of recycled water to irrigate food crops. The Order did not discuss perchlorates, address Wilson's comments, or contain any findings to support this conclusion.

2. Course of Proceedings

A hearing on the writ of mandate was held on July 28, 2015. The court denied the petition, holding that the Board was not required to make findings because the decision was quasi-legislative, not adjudicative. Even if the Board was required to make findings, the Board's finding that the public health would be protected by compliance with the Recycling Criteria was sufficient to satisfy the statutory requirement that it consider the need to prevent nuisance.

Judgment was entered on August 17, 2015.

B. Applicable Law

A new trial is a re-examination of an issue of fact in the same court after a trial and decision by a jury, court or referee. CCP §656. CCP section 657 sets forth the grounds upon which a party can seek a new trial. They are as follows: (1) irregularity in the proceedings of the court, jury or adverse party, or any order of the court or abuse of discretion by which either party was prevented from having a fair trial. (2) misconduct of the jury; and whenever any one or more of the jurors have been induced to assent to any general or special verdict, or to a finding on any question submitted to them by the court, by a resort to the determination of chance, such misconduct may be proved by the affidavit of any one of the jurors; (3) accident or surprise, which ordinary prudence could not have guarded against (4) newly discovered evidence, material for the party

making the application, which he could not, with reasonable diligence, have discovered and produced at the trial; (5) excessive or inadequate damages; (6) insufficiency of the evidence to justify the verdict or other decision, or the verdict or other decision is against law; and (7), error in law, occurring at the trial and excepted to by the party making the application.

Whenever the court grants a new trial, it shall specify the ground or grounds upon which it is granted, and the court's reason(s) for granting the new trial upon each ground must be stated. CCP §657. Furthermore, a new trial shall not be granted upon the ground of insufficiency of the evidence to justify the verdict or other decision, nor upon the ground of excessive or inadequate damages, unless after weighing the evidence the court is convinced from the entire record, including reasonable inferences therefrom, that the court or jury clearly should have reached a different verdict or decision. Id.

A party intending to move for a new trial must file with the clerk and serve upon each adverse party a notice of his intention to move for a new trial, designating the grounds upon which the motion will be made and whether the same will be made upon affidavits or the minutes of the court or both, either: (1) before the entry of judgment; or (2) within 15 days of the date of mailing of notice of entry of judgment by the clerk of the court pursuant to CCP section 664.5, or service upon him by any party of written notice of entry of judgment, or within 180 days after the entry of judgment, whichever is earliest. CCP §659. Upon the filing of the first notice of intention to move for a new trial by a party, each other party has 15 days after the service of such notice to file and serve its own notice of intention to move for a new trial. Id.

Within ten days of filing the notice of intention to move for a new trial, the moving party shall serve upon all other parties and file any affidavits intended to be used upon such motion. CCP §659a. Other parties shall have ten days after such service within which to file and serve upon the moving party any counter-affidavits. Id.

In ruling on a motion for new trial on a cause tried by the court without a jury, the court may, on such terms as may be just, change or add to the statement of decision, modify the judgment, in whole or in part, vacate the judgment, in whole or in part, and grant a new trial on all or part of the issues; or, in lieu of granting a new trial, may vacate and set aside the statement of decision and judgment and reopen the case for further proceedings and the introduction of additional evidence with the same effect as if the case had been reopened after the submission thereof and before a decision had been filed or judgment rendered. CCP §662.

C. Analysis

Petitioner Wilson moves for a new trial on the grounds that there was insufficient evidence to justify the decision, the decision is against law, and an error of law occurred at the trial. Petitioner seeks a grant of his motion unless the Board consents to a modified judgment submitting his comment about perchlorate to the California Department of Public Health ("CDPH"), solicit CDPH's view, and exercise its sound discretion for the general permit. Mot. at 2.

1. Timeliness

A notice of intention to move for new trial must be filed and served upon the parties of record not more than 15 days following the notice of entry of judgment. CCP §659. Within ten days of the filing of the notice of intent to move for new trial, the moving party must serve and file such affidavits (and authorities) on which the moving party intends to rely. CCP §659a.

In the instant case, judgment was signed and entered on August 17, 2015. The clerk gave notice of entry of judgment on August 17, 2015. The notice of intention to move for new trial was timely filed on September 1, 2015, exactly 15 days after the judgment was signed and entered. The October 15, 2015 hearing date is within the sixty days for hearing a motion for new trial prescribed by CCP section 660.

2. Merits[1]

a. Duty to Consult

Petitioner Wilson argues that the Board has a duty to consult with CDPH whenever the evidence shows a public health issue where the answer is not clear cut. Mot. at 5. He notes that regional water boards (not the Board) are required to consult with CDPH prior to issuing permits for recycled water use. Water Code §§ 13523, 13523.1. In 1996, CDPH and the Board entered into an agreement (the MOA) delineating their respective obligations. The agreement provides that the CDPH is the primary agency responsible for public health protection, and the Board has consistently relied on the expertise of CDPH for establishment of permit conditions. Mot. at 5-6.

Petitioner notes that the court's decision relied on the presumption that official duty has been regularly performed under Evidence Code section 664, and held that Petitioner had to show that the perchlorate articles he relied upon were unknown when 22 CCR section 60304 was promulgated by CDPH. Petitioner describes this burden on an ordinary citizens "absurd." Mot. at 7. He argues that 22 CCR 60304 was last revised in 2000 and he submitted articles that were published after that date. Mot. at 7-8. He contends that this suffices to compel the Board to investigate further by contacting CDPH, and the presumption that CDPH evaluated perchlorate contamination is inapplicable because CDPH has no duty to evaluate all contaminants, particularly those that are harmful only because they accumulate in plants on a variable basis depending on climate. Mot. at 9-10. Therefore, the court cannot conclude that CDPH had a legal duty to include protection against what Wilson calls "systemic risks", which should be addressed by local or regional recycling criteria depending on climate. Mot. at 10.

Arguing a procedural defect, the Board contends that Wilson brought his petition for administrative mandamus under CCP section 1094.5 and now Wilson is seeking to compel it to comply with an alleged mandatory duty, which requires a writ of traditional mandamus under CCP section 1085. *See* Rodriguez v. Solis, (1991) 1 Cal.App.4th 495, 501-02. In order for the court to issue an amended judgment with the requested order requiring the Board to consult with CDPH, The Board argues that Wilson would need to amend his petition to state a new cause of action under CCP section 1085. Opp. at 1-2.

Wilson claims that he is not attempting to enforce a mandatory ministerial duty, characterizing the Board's duty as a duty to receive evidence of CDPH's views on Wilson's comments. Reply at 5. He notes that the Legislature has provided administrative mandamus as

---

[1] Petitioner asks the court to judicially notice five exhibits, two documents from the court file, a reporter's transcript from the July 28, 2015 hearing, a February 20, 2996 memorandum of agreement between the Department of Health Services and the State Water Resources Control Board, and various provisions of Title 22 CCR. The court documents and reporter's transcript need not be judicially noticed. The court judicially notices the memorandum and CCR provisions. Ev. Code §452(b), (c).

the sole remedy to set aside a general permit. Water Code §13330. Reply at 4.

Wilson is correct that he is required to proceed under administrative mandamus and not by traditional mandamus, but he is not correct that he is not attempting to enforce a mandatory, ministerial duty. A duty to consult is a procedural requirement, not a substantive one. The court will evaluate Wilson's argument in the context of his administrative mandamus claim. The question is whether the Board had a mandatory statutory or constitutional duty to consult with CDPH, and whether the Administrative Record shows non-compliance with that duty such that it was a failure to proceed in the manner required by law and an abuse of discretion.

The Board did not have a mandatory statutory duty to consult with CDPH before adopting the general WDRs. The Board issued the General WDRs pursuant to Water Code section 13263(i), which does not require any consultation. The provisions relied upon by Petitioner – Water Code sections 13523 and 13523.1 – concerning a regional board's issuance of a "water reclamation requirement" and a master reclamation permit, respectively – do not apply to the Board or a general WDR. The MOA between the Board and CDPH does not require consultation, and a contract cannot support a mandatory duty anyway because mandamus cannot compel the exercise of discretion. State v. Superior Court, (1974) 12 Cal.3d 237. Any party, including a public agency, has the discretion to follow or breach a contract (with consequences of course). Moreover, the MOA's purpose is to assure that the parties' authority would be exercised in a coordinated and cohesive manner, and the Board's deference to the CDPH's actions in 22 CCR section 60803 fulfills that goal. The Board had no mandatory duty specifically to consult CDPH over perchlorate levels in recycled water.

In any event, Wilson's argument that the Board failed to consult with CDPH before issuing the General WDRs is, as Wilson admits, a new argument that Petitioner did not present to the court at the trial. A legal argument may be raised for the first time in a new trial motion "so long as the new theory presents a question of law to be applied to undisputed facts in the record." Cal Sierra Construction, Inc. v. Comerica Bank, (2012) 206 Cal.App.4th 841, 851. But as the Board points out (Opp. at 2-3), evidence of discussions between the Board and CDPH were not included in the Administrative Record because they were not relevant to the issues raised by the petition. Wilson's argument concerning the Board's duty to consult CDPH is therefore procedurally defective as not based on undisputed facts from a pertinent administrative record.

The Board now provides evidence that it did consult with the CDPH prior to adopting the General WDRs. Board staff met and conferred with CDPH staff, received CDPH input on the draft General WDRs, and revised the General WDRs based on CDPH comments. Farahnak Decl. Exs. A-E. None of CDPH's suggestions were regarding additional monitoring requirements for irrigated agriculture or perchlorates. Farahnak Decl. ¶6. Wilson objects to this evidence as outside the Administrative Record. This objection is valid, but merely proves the procedural defect in his argument.

Wilson also argues that these discussions between CDPH and the Board did not relate to Wilson's public comments about perchlorate, and the Board was required to specifically seek CDPH's opinion on Wilson's comments. Wilson provides no support for this argument, for it would require consultation with CDPH anytime a public comment raises a reasonable issue of public health.

As for Wilson's argument that the evidentiary burden imposed by the court under Evidence Code section 664 is unreasonably high because an ordinary person could never be expected to

include evidence of what CDPH had before it when it adopted 22 CCR section 60304, he misunderstands the court's point concerning the Board's duty when a citizen comments on a proposed WDR. The court merely stated that it must presume CDPH did its job when it promulgated 22 CCR section 60304, which means that it addressed any extent public health issues. In order to attack the Board's reliance on CDPH's regulation, Petitioner had to undermine it by showing that the perchlorate issue was unknown to CDPH when it promulgated 22 CCR section 60304. This is not an issue of unreasonable burden, but rather the viability of CDPH's regulation and the Board's right to rely on it. CDPH was required to adopt uniform statewide recycling criteria under Water Code section 13521, and Wilson's argument that local and regional regulation is more appropriate because orchards in desert and coastal climates will accumulate perchlorate at different levels again is a policy argument unsupported by law.

Wilson's contention that the Board has a duty to consult with CDPH is not grounds for a new trial.

b. Conflict with Topanga

Petitioner claims that the court's decision that general WDRs need not be supported by findings conflicts with Topanga Association for a Scenic Community v. County of Los Angeles, ("Topanga") (1974) 11 Cal.3d 506. Wilson notes that Water Code section 13330 is an independent legislative mandate requiring the application of CCP section 1094.5, and contends that the court's quasi-legislative/quasi-judicial analysis is not germane. He argues that there is nothing inherently wrong for a legislature to require an agency to make findings in a proceeding that has legislative characteristics. Once it is determined that CCP section 1094.5 applies, Topanga also applies. Mot. at 12.

Wilson's argument is inconsistent with the purpose of a new trial motion, which is that the error must be prejudicial in that, if changed, it could affect the case outcome. Bristow v. Ferguson , (1981) 121 Cal.App.3d 823, 826. Wilson acknowledges that the court ruled that the Board's findings were sufficient even if Topanga did apply. Mot. at 11-12. Thus, even if the court were inclined to reconsider its holding, any new trial would be meaningless because the outcome would be the same. *See* CCP §475. This is not an appropriate ground on which to grant a new trial.

Wilson claims that the court's decision will fundamentally change how Board hearings will be conducted and would be *res judicata* for any future WDR proceedings in which he makes comments to the Board. Reply at 10. The short answer is that a court decision's potential *res judicata* effect on a future ruling is not a basis for new trial. Moreover, the Board agrees that the court's decision on the Topanga issue will not bind its procedure in making findings in future proceedings. Opp. at 8.

D. Conclusion

Petitioner Wilson's motion for a new trial is denied.

EXHIBIT 11

COURT OF APPEAL OF THE STATE OF CALIFORNIA

SECOND APPELLATE DISTRICT

DEPARTMENT 85 HON. JAMES C. CHALFANT, JUDGE

ANDREW C. WILSON,	)	
PETITIONER,	)	
vs.	)	NO. BS149632
	)	
STATE WATER RESOURCES CONTROL BOARD,	)	
RESPONDENT.	)	

APPEAL FROM THE SUPERIOR COURT OF LOS ANGELES COUNTY

HONORABLE JAMES C. CHALFANT, JUDGE PRESIDING

REPORTER'S TRANSCRIPT ON APPEAL

TUESDAY, JULY 28, 2015

THURSDAY, OCTOBER 15, 2015

VOLUME 1 OF 1

PGS. 1 THROUGH 50

BUFORD J. JAMES, CSR 9296

OFFICIAL REPORTER

SUPERIOR COURT OF THE STATE OF CALIFORNIA

FOR THE COUNTY OF LOS ANGELES

DEPARTMENT 85 HON. JAMES C. CHALFANT, JUDGE

ANDREW C. WILSON,	)	
PETITIONER,	)	
vs.	)	NO. BS149632
	)	
STATE WATER RESOURCES CONTROL BOARD,	)	
RESPONDENT.	)	

REPORTER'S TRANSCRIPT OF PROCEEDINGS

TUESDAY, JULY 28, 2015

FOR PETITIONER: ANDREW C. WILSON, IN PRO PER

FOR RESPONDENT: JEFF STOCKLEY, SUPERVISING DEPUTY ATTORNEY GENERAL AND
ERIC M. KATZ, SUPERVISING DEPUTY ATTORNEY GENERAL

BUFORD J. JAMES
OFFICIAL REPORTER 9296
111 NORTH HILL STREET
LOS ANGELES, CALIFORNIA 90012

M A S T E R I N D E X

CHRONOLOGICAL LIST OF WITNESSES

WITNESSES	DIRECT	CROSS	REDIRECT	RECROSS
(NONE CALLED)				

ALPHABETICAL LIST OF WITNESSES

WITNESSES:	DIRECT	CROSS	REDIRECT	RECROSS
(NONE CALLED)				

EXHIBITS

EXH. NO.:	FOR IDENTIFICATION	IN EVIDENCE	WITHDRAWN
(NONE OFFERED)			

CASE NUMBER: BS149632
CASE NAME: WILSON VS. WATER CONTROL BOARD
LOS ANGELES, CALIFORNIA TUESDAY, JULY 28, 2015
DEPARTMENT 85 HON. JAMES C. CHALFANT, JUDGE
REPORTER: BUFORD J. JAMES CSR 9296
TIME: 9:30 A.M.
APPEARANCES: (AS NOTED ON TITLE PAGE)

--o0o--

THE COURT: Wilson versus State Water Resources Control Board, BS149632.

MR. KATZ: Good afternoon, your Honor, Supervising Deputy Attorney General Eric Katz for the respondent, State Water Resources Board.

THE COURT: Counsel.

MR. WILSON: Good afternoon, your Honor, Andrew Wilson petitioner, in pro se.

THE COURT: Good afternoon.

This is here on Mr. Wilson's petition for administrative mandamus to compel the State Water Resources Control Board to set aside his order for general waste discharge as a permit, General Waste Discharge Permit, adopted on June 3rd, 2014.

There are two issues presented by Mr. Wilson. One is the order is not supported by the findings because there is no finding about perchlorate which he presented evidence and articles that perchlorate,

one, is harmful to humans, most importantly, pregnant women; two, that it does not dissipate in water supply and, in fact, accumulates in orange groves, orange trees, such that orange trees can have higher perchlorate levels than waste water because they take up the perchlorate with irrigation water and then concentration accumulates. Actually, accumulation and concentration are the same thing. And the order says nothing about that; therefore, the order is not supported by the findings and then the findings are not supported by substantial evidence.

While Mr. Wilson presents an interesting issue, the fact is that this general permit, I think, is quasi-legislative in nature because it's -- I mean, I conceptually, think it's being issued to a whole host of permittees, none of whom were before the Board when it issued its order.

And for that reason, I think -- I agree with Mr. Wilson that the statute says 1094.5 applies and 1094.5 review is customarily -- Topanga applies to that because it's a quasi-adjudicative decision, I don't see how you can adjudicate an issue where the applicants are not even in front of you. I view this as quasi-legislative in nature, which is important because Topanga doesn't apply in quasi-legislative decisions.

In any event, if Topanga does apply, I agree with the Board that all it has to do to satisfy Topanga is to make findings that support the conclusion that the legislative requirements for general WDR's in the water

code have been satisfied, and the Board's order did that.

So then the question is is there substantial evidence. Mr. Wilson argues that the Department of Public Health regulation that says this is how you clean the recycled water for use in orange groves was issued 15 years ago and does not specifically mention perchlorate. His articles are more recent, the implication being that the more recent articles give new-found information. Well, you haven't shown that.

But, in any event, this is an issue of substantial evidence. The issue is not whether your evidence is better, but whether the Board had substantial evidence on which to rely. The expertise of another agency is certainly substantial evidence. There is no question the Department of Public Health had the expertise. They issued the regulation.

Mr. Wilson may not like the regulation, and it doesn't specifically mention perchlorate, but the truth is it doesn't mention any particular contaminant. Rather, it explains the process by which recycled water treatments for orchard irrigation may occur. And implicit in that is that the agency addressed all potential contaminants and uses of recycled water, including perchlorate contamination when it did so, and it's presumed to have done so.

And so there is substantial evidence. It may not be, per Mr. Wilson, good evidence, but it's substantial. So the tentative is to deny.

Have you seen it?

MR. WILSON: Yes, I got it.
THE COURT: You wish to be heard?
MR. WILSON: Yes.
THE COURT: Go ahead.
MR. WILSON: May I be seated?
THE COURT: You may.
MR. KATZ: And may I?
THE COURT: Yes.
MR. WILSON: Before I start, your Honor, could you give me an idea of how much time you want me to talk or willing to have me talk?
THE COURT: I don't want to put a governor on you, but I'm not feeling well. So if you could truncate it, I would appreciate it, but I don't mean to cut you off.
MR. WILSON: Yes. I'll just try to hit some high points on the tentative that jumped out at me as I read it.
I know you said that it's most probably quasi-legislative, and you ruled, obviously, in the alternative that satisfies Topanga. And that's the real issue for me, is whether it satisfies Topanga.
THE COURT: I mean, I don't get a sense for how this hearing was conducted, but I assume the applicants weren't there. They issued a general order, and you apply later on. Isn't that how it work?
MR. WILSON: That is how it works, except that actual applicants apply later on, but at the hearing you have advocates on both sides of the issue. You have people that represent the sewer plants that were trying to get the

order weakened, and you have environmentalists trying to get the order strengthened. So they are on both sides.

THE COURT: Let me ask the counsel for the Water Board. What do you think?

MR. KATZ: Well, it was conducted as a normal noticed hearing. It wasn't a rule making process.

THE COURT: But a noticed hearing can be quasi-legislative, certainly. Rule making would be flat-out legislative. You know, it depends on whether there is somebody who is required to present evidence in opposition.

I mean, all I can look at is, by way of parallel, is the CEQA case in which there is an applicant who appears, people oppose. And, you know, that's 1094.5 because it's -- actually can be either quasi-adjudicative or quasi-legislative. I don't think it matters too much, and it may be I'm going off the deep end here. It's clearly 1094.5, governed by 1094.5. Whether that means Topanga applies, I'm not convinced that Topanga applies to all 1094.5 hearings, judicial review hearings. Although, maybe it does.

MR. WILSON: Can I comment?

THE COURT: I'm certainly -- Yes. It's certainly possible I'm wrong there. Let's put that way.

MR. WILSON: I would just like to respectfully suggest that you are wrong because Topanga is an interpretation of the language of 1094.5.

THE COURT: It is.

MR. WILSON: And when an order -- when a statute says that a certain order will be governed by a 1094.5, you can't -- I don't think you read a statute to say, well, this order is somehow severable and part of it will be governed by Topanga and 1094.5 and part of it won't be governed.

THE COURT: Yes, you might be right. It might be that the legislature when it passed whatever the statute is, the water code provision, was not thinking about general permits; it was thinking about individual permits when it wrote that language saying that be governed by 1094.5.

MR. WILSON: Well, I think it was thinking about both, because permits, historically, are considered to be quasi-adjudicative and --

THE COURT: Individual permits, certainly.

MR. WILSON: Yeah. Well, this is a permit with a lot of permittees, but as I read your opinion the theory you were thinking it was -- it was just quasi-legislative because it had future application, but all permits have future application.

THE COURT: No, that wasn't my theory. I agree with you that when they do apply, once this order is passed, it's a ministerial duty. If they meet the requirements, boom, they get their permit. I agree with you on that.

But what I was thinking is that this is a situation broad in -- We have no idea how many permittees

there are going to be. There could be tens of thousands of permittees. It seems like it's broad in scope and not the kind of permit to which a Topanga analysis -- you know, it seemed pretty quasi-legislative in that regard, but I -- I could be all wet. Because you are right, Topanga interprets 1094.5. This is a 1094.5, other than the Government Code, I guess it was.

MR. KATZ: For what it's worth, your Honor, when I got the case I thought quasi-legislative, Topanga, you know, doesn't apply. Case closed. But at the same time in speaking with the Water Board they recognize that it's a mixed issue, and there is, I think -- I think from the Water Board's point of view, there is no clear answer, but that they do -- they proceeded as if it was an adjudicative proceeding.

They for General Order, for purposes of ex parte communications on General Orders, they apply adjudicative procedures for those ex parte communications in an abundance of caution. So I think it -- I mean, it's an interesting issue, but I guess I come down where the Court did, that it's not necessary to resolve for this case because, if Topanga applied, the Board complied with it.

THE COURT: I do agree with that.

MR. WILSON: I'll leave that issue, except for one final statement. You know, there is the Administrative Procedure Act, the tier one and tier two, and the State Board has issued, you know, advisory opinions on their other website saying that these types of orders are

adjudicative proceedings under that --

THE COURT: When you say that, you need to distinguish between individual permits and general permits. They say both?

MR. WILSON: They say both, yes. I do want to say that when I first sat down to do the writ, I was thinking along your lines, what is this thing. And it wasn't -- I had the same feelings you did, but when I got into the statutes and the website and analyzed the law, I came in positively on 1094.5. I didn't do it in the alternative. I said this is it. That's where I am coming from.

THE COURT: It's a good argument. That's all I can say. I don't know who is right.

MR. WILSON: Did Mr. Katz want to say something on that point?

MR. KATZ: No.

MR. WILSON: I want to go on to -- it seems like on the bottom of page 9 of your order you talk about an agency is presumed to have regularly performed its official duties.

THE COURT: Right.

MR. WILSON: And you cite that Evidence Code.

THE COURT: Right.

MR. WILSON: And that the Department of Public Health must be presumed to have done its job in issuing the regulation and considered all potential contaminants and uses of recycled water including perchlorate contamination.

THE COURT: Right.

MR. WILSON: I'm not sure where they mean all there. Because in the record at page 267, Mr. Bishop from the Water Board is talking about these constituents in recycled water that are of concern.

THE COURT: Who is Mr. Bishop?

MR. WILSON: Mr. Bishop is a director at the Water Board. Let me get his exact title for you.

MR. KATZ: I don't know the exact title, but he's a high-level staff person.

MR. WILSON: Chief Deputy Director of the State Water Board. He says about half way down the page, "We will never be able to address the hundreds of thousands of potential chemicals."

THE COURT: Right. He's a Water Board guy. He's not a Department of Public Health guy.

MR. WILSON: That's right. He's a Water Board guy.

THE COURT: That's why the Water Board relies on the Department of Public Health, which presumably did address all -- maybe not all the hundreds of thousands, but they better have addressed all of the categories of potential chemicals that might be there.

MR. WILSON: I'm not sure what you mean by "categories."

THE COURT: Well, there are certain classes of chemicals in pharmaceutical needs that are related to each other; right?

MR. WILSON: Sure.

THE COURT: I'm thinking because I'm sick that Motrin is related to Advil and it's related to all other -- what they called NSAIDS, non-steroidal anti-inflammatories. They are all related. So you could look at classes of chemicals if they determine that those classes are appropriately made.

And I'm not talking about just two or three classes. They have to be presumed to have done their job, which means they looked at all chemicals that would be properly classified in a particular risk area, health risk area, and deemed these levels to be safe.

Doesn't mean they did it. You could disprove that they did that; although, I would think you would do that in front of the Department of Public Health. But all we have is that the Water Board says they are the experts. They passed a regulation. That regulation says do it this way to clean out any contaminates, and that's what we're adopting for our permits. That's really what happened, and they can do that.

MR. WILSON: I would like to say something about that.

THE COURT: Please do.

MR. WILSON: If they analyze these potential contaminants, there is at least two avenues they could come to a conclusion of that the contaminant itself wasn't harmful after extensive study, or they could decide that these treatment processes we have in place were eliminated.

THE COURT: Right.
MR. WILSON: Two ways to go.
THE COURT: Yes.
MR. WILSON: You can't tell what way they went.
THE COURT: No, you can't. Why do you get to complain about that in this proceeding?
MR. WILSON: I get to complain about it because this Title 22 regulation is their evidence.
THE COURT: Well --
MR. WILSON: It's not my evidence.
THE COURT: That's true. It is -- I mean, basically, the regulation says as long as you do this recycling cleaning you will be safe.
MR. WILSON: I would --
THE COURT: That's what it says.
MR. WILSON: I would just object. There is no word "this will be safe" in the regulation.
THE COURT: I'm paraphrasing.
MR. WILSON: There is no paraphrasing that says it's safe.
THE COURT: But I'm not sure what the point is.
MR. WILSON: My point is this: I am approaching this from the perspective that the Water Board at this hearing could have looked at my evidence or somebody else could have come in there with evidence, and they actually could have decided that, hey, Title 22 is not covering this.
THE COURT: Right. Could have.

MR. WILSON: Could have. In other words, Title 22 is not conclusive evidence that just wiped out everything against it.

THE COURT: I don't know if they need it to be conclusive, but they certainly could have said, "Whoa, this perchlorate issue is a new issue, right, a lot of new articles on it, and the Department of Public Health's regulation that wasn't -- nobody thought perchlorate was a problem back when this regulation was promulgated. We better look at this."

They could have done that, and they didn't. And you have to assume that they didn't because nobody told them what I just said, that it is a new issue that was not addressed by the Department of Public Health.

MR. WILSON: I would say, though, it's a separate agency. And the only evidence in front of the Water Board was the text of the regulation, and they brought it in as their evidence proving that it's safe. And you have to look at that regulation and decide did they say the treatment reduced units perchlorate or did they decide that perchlorate is not harmful, and I brought in evidence on both points.

THE COURT: I know you did.

MR. WILSON: So when the Water Board sits down and analyzes this, which they do, how come we can't find out. There should be findings. How do we know? I don't know what they did.

THE COURT: I really think that argument has to

be made to the Department of Public Health. I think that the Water Board gets to rely on the Department of Public Health's regulation. And if the Department of Public Health did not consider either that perchlorate was harmful or that the cleaning process, recycling process, does not take care of the problem, that's an argument that has to be made to the Department of Public Health. We have a regulation in place that the Water Board relied on.

The only question is was that substantial evidence of the Water Board's reliance for purposes of all contaminants in orchards including perchlorate. The answer is it is. It has to be. You have to be able to rely on other agencies' expertise when they pass a regulation.

MR. WILSON: I would just like to say without -- when you talk about -- I don't mean you. When we talk about relying on another agencies' expertise, and the presumption is that the agency issued that rule based on the evidence that was in front of it, and they made a decision that was appropriate based on that evidence, one of the things you suggest is that I am challenging that. I'm not. I am not saying they did a bad job.

I'm saying they never looked at this other evidence that I have. They never saw those articles. Those articles are evidence in my favor. To say, well, they decided that based on whatever evidence they had 15 years ago, they foreseeing the future these other stages would come out --

THE COURT: You are assuming there were no other

articles, that these articles are the only ones. You are making that assumption. You can't do that.

MR. WILSON: It's my -- what I am saying is they didn't see my evidence.

THE COURT: No, they didn't see your evidence.

MR. WILSON: And my data was collected after they made their decision.

THE COURT: Actually, some of it was. Some was collected before they made their decision.

MR. WILSON: Well, the stuff -- the articles --

THE COURT: The articles were all written after the regulation.

MR. WILSON: And they didn't see the articles.

THE COURT: They did not.

MR. WILSON: They couldn't think ahead and predict --

THE COURT: That doesn't mean they didn't see other articles.

MR. WILSON: Well, the evidence -- at least I always thought when they are coming up with rules they look at all the evidence and the blend and the ruling emerges from it.

THE COURT: Yeah.

MR. WILSON: So to say that -- in other words, there is a presumption here that they have addressed both points. In other words, my evidence hit both points, that perchlorate is dangerous and the treatment doesn't remove it.

THE COURT: Yes.

MR. WILSON: Now, the presumption that you are referring lead to the conclusion that the Department of Public Health decides both, perchlorate is not harmful and is removed.

THE COURT: I only need to do one, right, either it's not harmful or it is removed. So I don't know that you can assume, presume, that they addressed and found both where they only need to find one. Which they found, I don't know, but we can rely on them to do their duty. It's only a presumption. You can overcome the presumption, which you didn't do.

Because if you had shown that here is what the Department of Public Health had before it when it made its ruling on perchlorate and here is what I have now and, boy, this is a much more serious risk to public health than the Board thought, than the Department of Public Health thought, then you might be in a different situation. Of course, you would have had to present that to the Water Board.

MR. WILSON: I think that presenting the administrative record that was compiled 15 years ago is really part of the conditional facts that would even make it relevant. In other words, this is not my evidence. There is no foundation that the decision -- that the Department of Public Health even addressed perchlorate, even thought about it.

THE COURT: No what?

MR. WILSON: There is no evidence that they thought about it.

THE COURT: There is. There is a presumption in the Evidence Code.

MR. WILSON: But you said a little bit ago that there is no presumption that they addressed hundreds of thousands. Is that the presumption?

THE COURT: I think the presumption is they did their job. If they are required to address hundreds of thousands, then the presumption is they did.

MR. WILSON: I read it differently. I think their statutory obligation is to sit down -- you know, depending on the budget constraints. They don't have unlimited army of people. They are supposed to sit down and think about from what they know and the evidence they have access to, hit the high points, then issue rules that deal with it.

And they did -- you know, one of the things that I would like to say is that I was making the point Title 22, not just 6034, doesn't mention perchlorate, but the whole thing doesn't mention perchlorate. And the actual definitions of what these treatments address is spelled out. The constituents are spelled out in the definitions. And the order says that the Section 60 of Title 22 doesn't expressly mention any recycling water contaminants. That's wrong.

THE COURT: Well, nobody gave me Title 22, which is a problem. You can't assume that I am going to look up

Title 22 on my computer. So you need to give me the regulation when you cite it, but all I did was look at your paper and your quote of Title 22 60, whatever it is. In your quote of it, it did not address perchlorate.

MR. WILSON: But at the bottom -- right. It didn't address perchlorate or anything else.

THE COURT: Or any contaminants.

MR. WILSON: But if you look at the definitions, it does address the specific constituents they dealt with.

THE COURT: If you could have shown that the regulation that the Department was relying on did not consider perchlorate, then you would have an argument.

MR. WILSON: I guess my point is if you look at the text of it -- you know, the decision of the Department of Public Health isn't in somebody's mind somewhere. It's in the text of the rule that they pass.

If you look at the text of that rule and there is nowhere anything about perchlorate, how can there be a presumption that they decided perchlorates are not harmful, we don't know which, and the Water Board is looking at my evidence and the Department of Public Health. They don't know what the Department of Public Health decided or which way to go. They decide the presumption anyway. Petitioner's evidence, you know, doesn't matter. It's overcome, but they don't really make a finding which part of my evidentiary tree they don't agree with.

THE COURT: Well, "they," the Water Board or, "they," the --

MR. WILSON: The water Board.

THE COURT: No, they didn't, nor do they have to. You presented evidence, and I don't know that you can assume they ignored it, but they certainly didn't address it in their order. That much is true.

You know, I don't want to argue for you, Mr. Katz. Why don't you take over the argument.

MR. KATZ: Well, your Honor has done a fine job. We agree with the findings in the tentative that a state agency is entitled to rely on the findings of another state agency when that state agency is -- has particular expertise.

The Water recycling criteria that we've been talking about was adopted by CDPH pursuant to a legislative mandate and Water Code Section 13520 and 13521, and I won't read it all, but the recycling -- the direction to CDPH was to adopt recycling criteria which will result in recycled water safe from the standpoint of public health for the uses to be made. And that's the -- that is what CDPH did.

And it's fair for the State Water Board to rely on their conclusion that it is safe from a public health standpoint to use the four categories of recycled water to irrigate orchards and for other uses as set forth in all of the recycling criteria adopted by CDPH.

THE COURT: So the Water Board is a subsidiary of the California Environmental Protection Agency; is that right?

MR. KATZ: Correct.

THE COURT: I didn't even know California had an environmental protection agency. And the Environmental Protection Agency, which branch in government, executive, independent, legislative?

MR. KATZ: Executive.

THE COURT: They take their orders from the governor.

MR. KATZ: Correct.

THE COURT: What is the bearing on this case that the governor told the Water Board to adopt general WDR's that meet the standards set by CDPH? Do they have any discretion to go outside the standards set by CDPH?

MR. KATZ: I suppose they could, but it's direction from the Executive as to what they should do, and it's certainly not an unreasonable direction to the Board to do that.

It's consistent with how CDPH and the State Water Board have been co-regulating recycled water since the MOU in 1996 was adopted that basically said -- the two agencies got together and said we both have responsibilities. CDPH, according to the legislature, your responsibility for recycled water is to adopt criteria from the standpoint of public health. The State Water Board's responsibility is to regulate recycled water from the standpoint of water quality. And they both work together in ensuring that the actions of both are consistent with the other's.

THE COURT: Well, what if Mr. Wilson had shown

that perchlorate is a new problem, nobody knew about it until two years ago? It's a serious public health problem. And he also showed that the Department of Public Health did not consider perchlorate when it issued it's regulations. Would the Water Board have been bound by the Governor's direction, or could it consider perchlorate issues?

MR. KATZ: Well, that's interesting. It would be inconsistent with the MOU to act --

THE COURT: Is the MOU between the two agencies?

MR. KATZ: Correct. It would be inconsistent with the MOU for the State Board to take a contrary public health position to the Department of Public Health, and I believe there is a dispute resolution process in the MOU if there is a disagreement.

THE COURT: Well, arguably, by my hypothetical, there wouldn't be any disagreement. The Department of Public Health would have never considered perchlorate. They just didn't know about it when they issued their regulation so it wouldn't be inconsistent.

MR. KATZ: Well, I guess there is also a third option of why it is that the Department of Public Health did not require monitoring for perchlorate for these particular types of uses, and that is the possibility that DPH didn't believe that perchlorate would be present in recycled water in concentrations that were of significance.

THE COURT: Well, that is hole in your presentation, isn't it, Mr. Wilson, that perchlorate is in California's -- I don't know where your studies are from.

They are not California studies, are they?

MR. WILSON: Well, the evidence that I put in was it came from health product manufacturing that was using perchloric acid in the process.

THE COURT: It gets in the waste water.

MR. WILSON: It gets in the waste water.

THE COURT: I mean, how many industrial applications in California use perchloric acid? I mean, do we know, or is this something that's happening in New Jersey, for example, as opposed to California?

MR. WILSON: Well, the record does not have breakdown of who is using perchloric acid in California. I mean, I just -- I'm trying to -- I do think that your question about the Memorandum of Understanding, you know, could they have stepped in and required more stringent requirements in Title 22, and then Mr. Katz suggested, well, that might have violated their Memorandum of Understanding, but I think it's really a question of their statutory authority. Because their statutory authority allowed them to do it, and if they bargained that way with some contract, I'm not sure he's saying that.

THE COURT: I fully understand. And under my hypothetical I would expect them to do something. I would not expect them to say, "Well, we'll just rely on their regulation," would not expect them to do that. Whether their Memorandum of Understanding --

MR. WILSON: Whatever it says.

THE COURT: Yes.

MR. WILSON: Right.

THE COURT: But that's not what we have here, and I do think I get to rely on it. Remember, we're talking only about substantial evidence, not whether an independent review of the evidence says there might be a problem here, which is what your evidence presents, but, rather, is there substantial evidence, whatever, however good your evidence is, is there substantial evidence for the Board to -- for the Water Board to rely on, and there is.

MR. WILSON: The other thing that really bothers me about that, I have a science background. I look --

THE COURT: I do too, by the way. That was a long time ago.

MR. WILSON: Well, mine was a long time ago. I've forgotten most of it.

THE COURT: Me too.

MR. WILSON: It just galls me to look at these treatments and suggest things that get perchlorates out of the water. It just galls me. Everybody knows perchlorate is like a dissolved salt ion. Desalinization is a big process. The idea you could run it through a sewer plant, you could hook up to the Pacific Ocean and desalinate, that anybody thinks these processes remove perchlorate, blows my mind.

THE COURT: Well, okay, don't below your mind yet because I don't know anybody has said that. What they have said is perchlorate is not a problem in recycled water in California. Why is it not a problem, as you pointed out,

they haven't said whether it's because there is no perchlorate in California or it's removed by the recycling process. So don't blow your mind that you know it can't be removed by the recycling process.

MR. WILSON: I'm sorry. I don't know why my mind shouldn't be blown. They didn't necessary say that. They might have said --

THE COURT: That there isn't any.

MR. WILSON: There is no perchlorate.

THE COURT: Nobody uses it. They use -- whatever.

MR. WILSON: But at some point don't we have to come back and have some findings of what is in the Water Board's mind on this?

THE COURT: I think you have got to go to the Department of Public Health. I'm not discounting the issue you have raised. It is an issue. I don't know where it goes. I don't know how important or significant it is, but public health is public health. It's an important thing. And, you know, I'm not suggesting that you should drop this issue, but I do think you've got to present it to the entity whose job it is to address this.

And I'm not saying the Water Board doesn't have a responsibility for public health. I think they do, but the primary entity that has that responsibility is the Department of Public Health, and you should present it to them.

MR. WILSON: Thanks for your words of

encouragement. You know, it makes me think that I am not total wacko.

THE COURT: First of all -- you know, the first thing -- I'll tell you, the first thing I do when I read briefs is are they well written, and your briefs were very well written, really good briefs. And the record looks exactly the way I want it to look. So, I mean, this was a perfect case for me to decide in terms of, you know, procedure. It was a pleasure.

MR. WILSON: Well, thanks. Can I make one final point because I think you are getting to maxed.

THE COURT: Yeah. I am actually starting to rally here, feeling a little better.

MR. WILSON: All right. I don't -- you know, whenever I am making these arguments, I don't mean in any way to -- I guess I am attacking what the thing says.

THE COURT: Yeah. That's what it's for. I became a lawyer and then a judge because I like to argue. It does not hurt my feelings for you to argue with me.

MR. WILSON: Okay. This statement about the presumption, and, you know, that the regulation addresses -- right before that cite, it says, "The regulation addresses the contaminants." I am at the bottom of page 9 quoting. "The regulation addresses the contaminates in recycled water could affect humans, and treatments are intended to protect public health from adverse impacts."

Now, the sentence doesn't continue and say

"adverse impacts from perchlorate." It stops with "adverse impacts" --

THE COURT: True.

MR. WILSON: -- in general. And the treatments are intended to protect public health from adverse impacts. The intention of these treatments and the regulation has to be derived from the text of the regulation.

THE COURT: Yes, but they don't have to address every chemical. As you pointed out, there are hundreds of thousands of chemicals.

MR. WILSON: Right. This seems to say that it addresses perchlorate. You can't pull that intent out of the text.

THE COURT: No, but it's a broad standard. And it's -- or umbrella, I suppose. Under the umbrella perchlorate fits. That's the way I look at it.

MR. WILSON: Then "an agency is presumed to have done its duties" and then "The Department must be presumed to have done its job in issuing regulations and considered all" -- we have been over that. This argument here is not in the Water Board's order.

THE COURT: It's not in the order. That's a Topanga argument.

MR. WILSON: That's something you drafted.

THE COURT: Yeah. I don't know whether you put it in your brief.

MR. WILSON: No, it's not in his brief.

THE COURT: It happens all the time that my

tentatives are my own thinking and may or may not track what somebody else has said.

MR. WILSON: Okay. This is leading up to my point. Topanga requires this stuff to be voted on by the Water Board, five members. They are supposed to have read this language and say I agree with that. They have never seen this.

THE COURT: Yeah. Well, what they have seen is that we're relying on Regulation 60305, or whatever it's called. The recycled criteria. Is that what it's called?

MR. WILSON: Right.

MR. KATZ: Yes.

THE COURT: That's all they need to see. They don't need to say, "and under Evidence Code 664 the Department of Public Health's regulation is presumed to be correct." They don't have to say that.

MR. WILSON: They -- well, I view that as a basis of their decision, that they do have to say, but that's my own, I guess, opinion.

I would close with saying we're sitting here right now, and I think that the -- the Water Board doesn't believe that the treatments remove perchlorate because of the salt ion. I think nobody thinks that.

THE COURT: I don't know. What is the Water Board's thinking? Are they thinking anything on perchlorate?

MR. KATZ: No, I think they are thinking that DPH did its job to determine what treatments are necessary and

what monitoring is necessary. Because that's really what the petitioner was asking for in the petition or in the comment letter, I believe, was to say you should be monitoring for perchlorate.

THE COURT: But the fact is the Governor declares emergency, we have a big drought, it's only getting worse. The Water Board is under a lot of pressure. Got to pass permits so we can recycling water, recycling is good. You know, could they have slipped over this in order to get permits out, sure they could have. Can one easily conceive of that happening, yes. Should they, no. I mean, they ought to protect public health as they are addressing the job problem, but we don't know.

I mean, it's all speculation. We got to have evidence. I have to have evidence in front of me. I can't speculate that, boy, this sounds bad, this perchlorate thing, let me remand to the Water Board so they can look at it again. I can't do that. I only call balls and strikes.

MR. WILSON: I --

THE COURT: I don't set policy.

MR. WILSON: I think as far as Title 22 being evidence, I don't think there is foundation that those treatments remove perchlorate, and I don't think that there is any foundation in the record that the Department thought perchlorate -- even considered perchlorate or concluded perchlorate was harmless --

THE COURT: See, you are mixing your records.

Whose record is that supposed to be in? It should be in the Department of Public Health's record. Doesn't have to be in the Water Board's record.

MR. WILSON: Yes, it does. The Water Board has to have evidence that's relevant to come into this record, relevant on the issue of perchlorate. Title 22 is their evidence. Is it relevant on the issue of perchlorate. I think there is no foundation.

THE COURT: Well, assuming not a foundation, but they don't have to lay one.

MR. WILSON: They do have to lay one.

THE COURT: No. Evidence Code 664 is -- the whole point of it is you don't have to lay the foundation. You say, boom, they have got a regulation, we presume it's correct. It's up to the petitioner to prove that it isn't.

So, no, they don't have to do anything more. That's the whole point of bypassing, you know, the building block of a foundation; otherwise, every agency would have to prove everything that every other agency does.

MR. WILSON: No. I think -- I mean, I think you have to -- you can say under 664 they did it right, but you have to -- I don't want to repeat myself endlessly. It's just we don't know. I think sitting here they think perchlorate is a problem, and I don't think they decided it wasn't a problem. I think -- in other words, they didn't do their job. They are supposed to weigh the evidence and make a finding, and they don't -- that doesn't show up in their record, the State Board's record.

THE COURT: I don't necessarily disagree with what you said, but at this level it's not whether they weighed the evidence. It's whether they have substantial evidence, and they do.

MR. WILSON: I would disagree. I think they have to consider all relevant evidence. Substantial evidence is not just one sided. They have to look at the whole picture.

THE COURT: I don't disagree with that. They do have to look at all the evidence and do have an obligation to weigh the evidence, but at this level, meaning in front of me, all I worry about is did they have substantial evidence, and they do.

Okay. I got to end this. I'm adopting the tentative. Please follow the last paragraph.

MR. KATZ: Thank you, your Honor.

MR. WILSON: Thank you, your Honor.

THE COURT: You want to waive notice?

MR. WILSON: Yes. I do want to thank you for the time you took.

THE COURT: Quite all right.

MR. WILSON: And being so receptive to argument.

I would also like to say Mr. Katz has, in terms of being on the other side as an attorney, was just great towards me. We had some difficult issues to work out in the scope of record which we disagreed on, and I just thought whenever you see him around the court house or anywhere, I would like all the judges to know he's really

good to work with.

THE COURT: That's good to hear. I think Department of Justice employees should always get that kind of affirmation.

MR. KATZ: If every pro per was like Mr. Wilson, the world would be a happy place.

THE COURT: All right. Thank you.

MR. WILSON: Thank you.

(Proceeding adjourned at 3:00 p.m.)

CASE NUMBER: BS149632
CASE NAME: WILSON VS. WATER CONTROL BOARD
LOS ANGELES, CALIFORNIA THURSDAY, OCTOBER 15, 2015
DEPARTMENT 85 HON. JAMES C. CHALFANT, JUDGE
REPORTER: BUFORD J. JAMES, CSR 9296
TIME: 9:30 A.M.
APPEARANCES: (AS NOTED ON TITLE PAGE)

--o0o--

THE COURT: Wilson versus State Water Resource Control Board, BS149632, number 3 on calendar.

MR. KATZ: Good morning, Your Honor, Eric Katz for respondent, the State Water Resources Control Board.

THE COURT: Mr. Katz.

MR. WILSON: Good morning, your Honor, Andrew Wilson, petitioner.

THE COURT: Mr. Wilson.

Okay. This is here on Mr. Wilson's motion for a new trial. I think there were three issues. The first is -- well, it's not an issue. The motion is timely made. The first issue on the merits is that Mr. Wilson argues that the Board, the Water Board, had a duty to consult with California Department of Public Health whenever the evidence shows that there a public health issue and the answer is not clear cut. So this is a duty to consult question. This is an issue that was not raised at the trial, and Mr. Wilson is raising it now.

There is discussion about whether he's actually seeking a mandatory ministerial duty and, if so, whether he's required to proceed by way of traditional mandamus. Mr. Wilson shows he couldn't proceed by way of traditional mandamus, and he is required to proceed by way of administrative mandamus.

And in my view the issue of administrative mandamus can be raised in that administrative mandamus context. So there is no procedural failure here in that regard.

However, mandatory duties are required to exist either by statute or constitutionally. You can't have a common law mandatory duty or an implicit mandatory duty. They have to be express. And, for example, in CEQA there are mandatory duties to consult. And this is not a CEQA case, and there isn't a mandatory -- it isn't CEQA, is it? No. And there isn't a mandatory, statutory, or constitutional duty to consult.

In any event, this is a motion for new trial which is a new argument, as Mr. Wilson admits, and a new argument can be made for the first time in a new trial motion only on undisputed facts in the record. Facts concerning consultation between the Board and the CDPH were not included in the record. So this is not the kind of issue that can be made in a motion for a new trial.

The Board presents evidence that it did consult. Mr. Wilson objected to that evidence as outside the record, which is well taken. He also argues that that

evidence doesn't show consultation about perchlorate, which is the issue that he is interested in. Nonetheless, all that does is show that this is not a proper argument for a new trial motion. Mr. Wilson also argues that the Board -- that the Court unfairly imposed a burden on him and all other citizens who contest the Board's actions by requiring him to show -- him and other citizens to overcome Evidence Code 664, which presumes that the Department of Public Health did its job when it promulgated 22 CCR 60304.

I'm not trying to impose a burden on Mr. Wilson or any other citizen. They are free to contend before the Board that its proposed permits, general permits, are not taking into account a public health issue, but the Board is entitled under that Evidence Code to rely on the Department of Public Health doing its job and the viability of the Department of Public Health's regulation.

If that regulation is not viable because new information has come out, then it is up to the citizen to present that information to the Department of Public Health, not to the Water Board, it seems to me. That was the point of the 664 presumption. It's not to increase the burden of citizens. Citizens raise issues, Water Board decides whether that issue requires some action, and the Water Board can rely on Evidence Code 664 and that the Department of Public Health did what its supposed to do. In this case it was obligated to adopt uniform statewide recycling criteria under the Water Code.

The last issue is a conflict with Topanga,

which Mr. Wilson acknowledges that my ruling was in the alternative, that is, I have found compliance with Topanga, even if it was initially found that it was not necessary to comply with Topanga. But if compliance was required, the Board did comply with it, and, therefore, it's not an issue that can be raised in a new trial motion because it would not change the outcome of the Court's decision.

Mr. Wilson argues that it could have a res judicata effect on future court proceedings in which he makes comment. And the answer is that potential res judicata effect on future rulings is not a basis for a new trial. This is a motion for a new trial. And, in any event, the Board agrees that it's not going to change its practice in making findings based on this Court's ruling.

So the tentative is to deny. Have you seen it?

MR. WILSON: Yes.

THE COURT: You wish to be heard?

MR. WILSON: Yes, please.

THE COURT: Go ahead.

MR. WILSON: May I be seated.

THE COURT: Yes, but you have to be done by noon. I'm sorry I'm going to have to cut you off at noon.

MR. WILSON: Okay. Just going back backwards from your last point.

The Board agrees that the Court's decision on Topanga will not bind its procedure in making findings in the future. I don't read that as an affirmative

statement by the Board that they are not going to follow the Court's decision. In other words, the Court has decided that findings are not required.

If they came forward and said, look, we acknowledge that's wrong, we're never going to follow the Court's decision, we're always going to follow Topanga, that would be one thing, but I don't hear them saying that.

THE COURT: I found that they did follow Topanga; therefore, they have a practice. And I assume that practice is to follow Topanga the way they see Topanga's requirements. And they intend to continue that practice.

Is that right?

MR. KATZ: Yeah. If I can speak to it. The issue of prejudice is whether there is prejudice in this trial that would require the Court to reach a different conclusion. The issue of prejudice that Mr. Wilson is raising is, well, will this ruling have adverse effects on him and others in future proceedings. And I'll provide the answer to that, but that's not the question of prejudice that the Court is supposed to look at.

THE COURT: Well, I understand. I indicated that, but he's addressing the last point, which is that you are not going to change your practice based on my ruling.

MR. KATZ: Well, I think, as a matter of law, the -- if in the next time the state Board adopts a general WDR, if it decides Topanga does not apply and it does not need to make findings and Mr. Wilson or anyone else in the public disagrees, they can bring a writ on that, and the

conclusion that the Court reached in this case isn't going to bind other trial courts.

You know, frankly, I don't know what the Board will do in any future proceeding because it was not relevant to resolve this motion.

THE COURT: Okay.

MR. WILSON: Okay. I just take that as being not any kind of promise about what the Board is going to do or not do.

THE COURT: I think that's right.

MR. WILSON: Yes. Okay. Well, there was obviously a ruling by the Court that the findings on -- that compliance with Title 22 insures that the water is safe, that that finding satisfied Topanga. That was in your order in the alternative. It did satisfy Topanga.

THE COURT: Right.

MR. WILSON: There was no finding as to -- the Board never made a finding on this issue, whether this duty existed. The basic fact under Evidence Code 664 was, and you brought it up in your ruling, there was never a finding by the Board that that duty existed.

And I tried to make the argument under Evidence Code 600(a). The presumption arises from the facts found or otherwise established in the action. Now, found would mean in a case where findings are required. That language, "or otherwise established in the action," that covers all the cases of jury trials or bench trials where findings are made. That would cover those cases.

But in this case I was arguing it wasn't a fact found and also it wasn't otherwise established by the evidence. And I assumed -- I was trying to argue it in the context of both because I knew you had this ruling out there that Topanga didn't apply. So, in a sense that the only -- you know, the question about whether this is a -- something I can raise now at new trial will have an effect on the case's outcome. I think it absolutely would have an affect on the case's outcome. You know, I can't get inside your head, but I think you approach that presumption of duty as if there was no requirement findings by the Water Board as to the existence of that duty.

THE COURT: Well, I do think that -- but, first of all, now your motion -- now you are arguing something that, A, you didn't argue in your new trial motion and, B, you never argued in your previous papers, which is that the noncompliance with Topanga is a noncompliance based on my analysis that 664 applies.

And the short answer is, one, that's not what you argued in your motion. You argued that I did find compliance with Topanga, and you argued that that fact should not conflict with your ability to contest it because it would be res judicata, that that's what you are arguing. So I did find compliance with Topanga, and I don't think you get to reargue the same issue to me again in your motion for new trial.

But, even if you can, the Board probably has no idea of what 664 of the Evidence Code is, nor are they

required to know. They, essentially, accepted its legal effect, which is, there is another agency that made a decision, and we rely on that other agency to do its job correctly, and, therefore, we are going to accept what we did and pull it into our permit approval.

That's -- they don't have to mention 664, but the legal affect of what they did is 664's legal effect. They certainly don't have to say that in a Topanga analysis, though.

MR. WILSON: Thank you. I want to go back to one other thing. When you went through the tentative and you talked about the burden, I was arguing that the burden was too high for an ordinary person to ever be expected to meet.

THE COURT: Yeah, I understand. And my point was I am not trying to impose that burden on you. It's more the effect, that is, you raise an issue. And let's assume you did exactly what your claim for a duty to consult. You raised an issue concerning perchlorate and public health where you believe the evidence showed the answer was not clear cut and, therefore, you contend that they should at least talk to the Department of Public Health about it.

And -- I forgot where I was going with that.

Oh, so I wasn't trying to increase your burden. You raise issues, and then the Board decides. It's the Board's decision that I'm trying to get at, not your burden as a citizen raising issues to the Board.

So do you understand what I'm saying? I'm

not saying you have to come armed to the Board hearing ready to prove that the issue was not adequately addressed by the Department of Public Health. I'm saying that the Board can rely on the Department of Public Health's regulation in deciding not to further address the issue that you raised.

You don't have any burden of proof at the Board hearing. All you do is raise issues. Then the Board decides what they want to do in the exercise of their discretion. That's what I was trying to say.

MR. WILSON: If I had met my burden or -- I don't want to call it a burden. If I had shown in front of the Board that I had undermined it showing that the perchlorate issue was unknown, you know, at the time California 2 was adopted, unknown to CDPH, what I was trying to get clarification on, was would then the Board have a duty to consult with CDPH, or no way never have a duty to consult with them?

THE COURT: I don't think -- I think the answer is there is no duty to consult unless it's statutory or constitutional. So since there is nothing that says they must consult, I would say they have no duty to consult.

However, a failure to consult where you have raised an issue of public health such that they should reasonably believe that the regulation they are relying on doesn't address it, then, yeah, they either better not rely on the regulation or consult. I mean, but that's a different issue.

MR. WILSON: Well, when I was focusing on that, if I had -- just like you had said, if I had made that showing, I'm arguing that, yeah, they couldn't rely on 22 and, yeah, they had a duty to consult. The question is like you were saying, where does the duty come from. It has to come from the statutes. It's implied in the statutory scheme --

THE COURT: There is -- there is no such thing as a implied duty to consult. It's got to be express. The duty to consult must be statutory, but failure to consult can be evidence that the reliance on the regulation was inadequate. That's what I am --

MR. WILSON: So this kind of gets to the same end point.

THE COURT: It could, yes.

MR. WILSON: It's a matter of semantics whether you frame it as a duty --

THE COURT: That's what lawyers do. Some would call it semantics. Others would call it law.

MR. KATZ: Your Honor, if I could jump in, I would think, following the Court's logic, that if petitioner has raised an issue that is called into doubt whether the regulation is substantial evidence, there could be any number of ways in which the Board could find --

THE COURT: Could address it.

MR. KATZ: -- other substantial evidence. One might be to go to CDPH and have CDPH say, "No, we thought about that issue and we dismissed it." The other would be

to find what other evidence would bolster --

THE COURT: Right. Do the leg work themselves and not rely on CDPH.

MR. KATZ: Exactly.

THE COURT: Yeah. They had options. If you had undermined their reliance on the regulation, then it would be up to the Board to decide what the best course would be. Could they consult with CDPH on the subject, sure they could. That would be one of their options.

MR. WILSON: The other option would be go hire their own expert and rely on him and forget CDPH.

THE COURT: Exactly.

MR. WILSON: I don't agree with that. I think that can't be right.

THE COURT: You are the one that has shown me that they both have public health responsibilities.

MR. WILSON: Absolutely.

THE COURT: And CDPH has primary public health responsibility. That's what you told me.

MR. WILSON: That's right. They absolutely do have primary responsibility.

I might have misheard what you said, but, as I read it, I'm bringing this evidence in that was an issue unknown to CDPH in my hypothetical. I'm bringing this evidence into the Board that was unknown, this issue of perchlorate was unknown to CDPH when they passed California 22. If I overcome the burden, they can't rely on Title 22. They either got to go to CDPH or the some other expert.

THE COURT: Yes. Right.

MR. WILSON: I don't have to also take that evidence as some kind of companion hearing to the CDPH; right. I'm talking about what is going on inside the Board.

THE COURT: No.

MR. WILSON: Okay. The other argument I wanted to make, and I know I'm running out of time, I just want to say that the whole point of this testing of perchlorate, the whole principle I was trying to get across, was that a use of recycled water can't be considered safe if a reasonable person would recognize that the degree of likelihood of adverse public health effects can't be determined without further testing or further investigation. That's just the basic principle I was trying to bring home.

THE COURT: I can't say that I disagree.

MR. WILSON: So when I -- when I make that showing, I didn't think it was appropriate to have to meet this burden -- I don't mean to call it a burden. My mind is blank and I can't think of another word, the burden of showing that they didn't consider it when they passed Title 22.

Because I think -- the example I wanted to kind of lay on you was suppose the safety level of perchlorate for Valencia oranges in the desert is one part perchlorate and the safety level Oxnard is five. I think that the Department of Public Health has to have the

discretion to say that, "Hey, you can use the water in Oxnard and not make them spend million of dollars for treatment to drag the perchlorate level down to one to make it something for something in the desert.

THE COURT: I don't know whether they have that authority or not. Maybe they do; maybe they don't. It depends on their authorizing statute, and the law is that an agency can only do what they're authorized to do, but they get to fill in the interstices of the statute with implicit authority so long as it's within the four corners, using your metaphor, the trunk of the orange tree, authorizing their action.

MR. WILSON: I -- see, I was thinking that the way you handled the presumption, you were saying, "Hey, they have an absolute duty to deal with that systemic risk uniformly statewide," meaning, they don't have a choice to have like regional --

THE COURT: I don't know what their statute provides. I only looked at the one statute that counsel cited to me that said they had their issue uniform criteria, but do they have the authority to make what you call a systemic analysis, I don't know.

But what I do know and what I told you before, you have every right to present this issue to the Department of Public Health. If they change their regulation, then you have every right to bring it back to the Water Board and say, look, the regulation you relied on has been changed.

MR. KATZ: Well, even more so, the general order says that General WDRs say that anyone that is seeking coverage under it has to comply with all of Title 22 regulations. So Title 22 changes because CDPH says, "Oh, this is a new risk, let's require monitoring." Well, we've never required it before. My assumption would be that that would automatically become a permit and, essentially, you know, ongoing deference to what CDPH believes is appropriate to protect public health.

MR. WILSON: I had just thought that when we were assuming they did their job and addressed all these risks, I thought it meant they addressed them in Title 22, that they put in requirements to make it safe.

THE COURT: Right.

MR. WILSON: So --

THE COURT: We did, but you are free to disagree with that. You are free to say, "Wait a minute, CDPH, I don't think you did address these risks. Look at these Articles I have. Changed your regulation." I'm not suggesting you shouldn't do that.

MR. WILSON: Right. I can do that, but I thought the assumption was that they did it so if a statewide -- statewide crier criteria, that meant that they set the rate at one, and they may spend million dollars down in Oxnard to treat their water.

THE COURT: Maybe. I don't know. I don't have any evidence before me what they did or didn't do.

MR. WILSON: But, I mean, the assumption is the

duty. The duty is that they had to address it in Title 22, all risks.

THE COURT: They don't have a duty to address unknown risks. Your whole argument is this was an unknown risk.

MR. WILSON: I was trying to work through a hypothetical that I came in there, you know, and showed that this is unknown an risk. In other words, the way I read your opinion was they had a duty to address all risks. Suppose perchlorate was a known risk. How could they have a duty to set the level at one statewide. That seems insanity.

THE COURT: I'm not sure I understand what you are saying. Set it at one statewide. Why would that be insane?

MR. WILSON: Because you force them in Oxnard to spend millions to treat the water that is otherwise safe.

THE COURT: You --

MR. WILSON: It's in their discretion.

THE COURT: How does that bear on your motion?

MR. WILSON: Because in your application of 664 of the Evidence Code you assume they had a duty to address all risks in Title 22. Address, i.e., make it safe.

THE COURT: Known risks, yes.

MR. WILSON: So how are they going to address perchlorate? Are they going to set it at one?

THE COURT: I have no idea how they address perchlorate.

MR. WILSON: That's the only way; right? You can't set it at five and have poison crops in the deserts. You are assuming they have to set it at one statewide, which is an incredibly wasteful result. I don't think the statute lends itself to that interpretation.

THE COURT: I have no idea.

MR. WILSON: But the assumption, though, of your duty, right, the duty -- they did their job. It's a statutory job. The statute says do your job, make it safe.

THE COURT: Yes.

MR. WILSON: Make it safe statewide.

THE COURT: Right.

MR. WILSON: Here comes perchlorate. You got to make that safe statewide. You set it at one or five, whatever you do, make it safe. That's your job.

THE COURT: If they have to be uniform, yes, they would have to go to the lowest one. Your argument is is that poor public policy. I'll accept that.

MR. WILSON: Statutory interpretation. I'm talking about we have to have a reasonable interpretation of the statute. And the way it's laid out in the order in your decision, it's not a reasonable interpretation.

THE COURT: What would be a reasonable interpretation?

MR. WILSON: That they have a discretion to not include systemic risk in Title 22 statewide criteria.

THE COURT: Maybe they do. And so?

MR. WILSON: If they have a discretion, then you

can't assume by doing your job that they addressed it. You see what I mean. They can leave it to a case-by-case analysis, leave it to regional criteria. It caves in the whole assumption.

THE COURT: That assumes facts not in evidence. I have no idea what they did. That's the whole point. We can't rely on -- and I've got to end you. I'm sorry.

MR. WILSON: One more point.

THE COURT: Yes.

MR. WILSON: On the record I -- 1094.5, as petitioner, I have a right to ask them to prepare all or part of the record. I asked them to prepare all the record. Every item that constituted all of the record. And that's not somehow undermined by whatever claims I put in my petition. I had that right for all of the record, and that's what I thought we were getting, all of the record.

THE COURT: So your argument is this consultation information that they have provided to which you have objected, if it really should have been part of the record, the certification -- and there would be a certification somewhere in the record that says this is either all or part of it. Did you look at the certification?

MR. WILSON: I confess that I don't know.

THE COURT: It will tell you whether they claim it's the whole thing or only part of --

MR. WILSON: That was at least my understanding, Mr. Katz, we're going for all the record.

MR. KATZ: I believe that's true, and the reason why the CDPH consultation documents didn't go in was not an affirmative belief that, "Oh, we're going to respond with a record only to his issues," but I think, as a practical matter, the Staff overlooked it not thinking that it was going to be --

THE COURT: Okay. So we have -- seems like I have a series of blunders by agencies. So we have a blunder by the Staff that they didn't include it in the record. Your understanding was this was the entire record. I'll accept that.

And so then we -- you know, to me it depends on what the certification says. If the certification says this is the entire record, then the Board is stuck with that fact. They can't mention or rely on this consultation evidence. On the other hand, if the certification says something less than that, then your -- you would argue that's not what I asked for. And I guess --

MR. WILSON: What I believed I was getting.

THE COURT: And what you thought you were getting. We're way beyond that here in a new trial motion.

MR. WILSON: I want to mention, anyway, they sort of restated my argument as a duty to consult, and my argument was more specific. Duty to consult and receive views about my -- that's what I was arguing about. It's undisputed in the record that they never -- the Board decision makers never received in evidence the views on --

THE COURT: I understood you to be arguing that

they did not consult about perchlorate. Yes.

MR. WILSON: Right. So that's an undisputed fact in this record.

THE COURT: I am not going to change the tentative. I understand your argument, and I don't have in front of me whether the record was complete or not complete on the certification. So the analysis on page 4 either is a correct analysis or not a correct analysis because I agree with you, if they told you you were getting the whole record, it says it's the whole record, they can't say now, whoops, there is evidence of consultation we should have put in. In any event, your point is it doesn't mention perchlorate anyway.

MR. WILSON: Right.

MR. KATZ: Had he raised the issue in his petition or the opening brief, the omission wouldn't have been made and we would have either put it or supplemented long before this post trial motion.

MR. WILSON: I have a question, Your Honor. Is the tentative -- is this thing part of this case file?

THE COURT: Yes.

MR. WILSON: C it will be stamped "filed"?

THE COURT: Yes. What happens, it will be filed. The minute order will refer to it as the order of the Court.

MR. WILSON: Okay.

THE COURT: So it is adopted as the order of the Court. You want to waive notice?

MR. WILSON: No, thank you. I would like notice.

THE COURT: All right. The Department is -- the Water Board is to give notice. I have to call this other case.

MR. WILSON: Okay.

MR. KATZ: Thank you.

(Proceeding adjourned at 12:08 a.m.)

SUPERIOR COURT OF THE STATE OF CALIFORNIA

FOR THE COUNTY OF LOS ANGELES

DEPARTMENT 85 HON. JAMES C. CHALFANT, JUDGE

ANDREW C. WILSON,	)	
PETITIONER,	)	
vs.	)	NO. BS149632
	)	REPORTER'S
STATE WATER RESOURCES CONTROL BOARD,	)	CERTIFICATE
RESPONDENT.	)	

I, Buford J. James, CSR 9296, Official Reporter of the Superior Court of the State of California, for the County of Los Angeles, do hereby certify that the foregoing pages 1 through 30 and page 31 through 50, inclusive, comprise a full, true, and correct transcript of the testimony and proceedings held in the above-entitled matter on TUESDAY, JULY 28, 2015 and THURSDAY, OCTOBER 15, 2015, respectively.

Dated this 4th day of February, 2016.

Buford J. James, Certified Shorthand Reporter

EXHIBIT 12

Fact Sheet

Frequently Asked Questions: General Order for Recycled Water Use

The proposed water reclamation requirements for Recycled Water Use (General Order) are intended to replace existing General Waste Discharge Requirements for Recycled Water Use (order WQ 2014-0090-DWQ) adopted by the State Water Resources Control Board (State Water Board) on June 3, 2014. The purpose of order WQ 2014-0090-DWQ is to streamline permitting of recycled water use statewide. The proposed General Order is intended to further encourage recycled water projects by acknowledging recycled water as a resource through water reclamation requirements, and allowing recycled water programs implemented in multiple Regional Water Board boundaries to be permitted by the State Water Board.

The following provides answers to frequently asked questions related to the General Order application process. More information on the use of recycled water is available at the State Water Board[1] or at any of the Regional Water Quality Control Boards.[2]

General Information

1. **What is recycled water?**
   Recycled water means water which, as a result of treatment of waste, is suitable for a direct beneficial use or a controlled use that would not otherwise occur and is therefore considered a valuable resource. Uniform Statewide Recycling Criteria (California Code of Regulations, Title 22, Division 4, Chapter 3) contains requirements for recycled water quality and wastewater treatment requirements for the various types of allowed uses. For nonpotable reuse applications, there are four types of recycled water based on levels of treatment: non-disinfected secondary, disinfected secondary-23, disinfected secondary 2.2, and disinfected tertiary. The level of treatment used is based on what the recycled water is intended for. Non-disinfected secondary recycled water is water with the lowest level of treatment, suitable for applications that have a very minimal public exposure level, such as irrigation for fodder crops. Disinfected tertiary recycled water goes through higher levels of treatment, sufficient for applications with more public exposure, such as irrigation of parks, decorative fountains, or artificial snowmaking for commercial outdoor use.

   A summary table -- courtesy of the East Bay Municipal Utility District -- showing various recycled water uses corresponding with minimum treatment levels is viewable at:

[1] Water recycling information is available at the State Water Board at http://www.waterboards.ca.gov/water_issues/programs/index.shtml#waterrecycling

[2] Contact information for the Regional Water Boards is available at http://www.waterboards.ca.gov/waterboards_map.shtml

https://www.ebmud.com/files/7614/3173/1139/recycled-water-uses-allowed-in-california-2013_0.pdf. The summary table is intended to be a visual aid, and is not to be relied upon as the State of California's representation of the law. Always refer to the published codes (Health & Safety Code or California Code of Regulations, Title 22) whenever specific citations are required.

2. **Can this General Order be used to permit recycled water production facilities?**
   No. Only distribution and use of recycled water is eligible for coverage under this General Order. Wastewater treatment facilities that intend to produce recycled water for reuse must obtain a separate coverage under a separate Regional Water Board permit. Wastewater treatment plants under 100,000 gallons per day (gpd) proposing to use recycled water can be covered under a statewide general Waste Discharge Requirements for Small Domestic Wastewater Treatment Facilities permit (Order WQ 2014-0153-DWQ).

3. **Is the Regional Water Board required to use the General Order?**
   The General Order is intended to be the primary method for Regional Water Boards to permit recycled water use. However, Regional Water Boards may determine a proposed use is not consistent with the General Order requirements or antidegradation analysis. In those cases, the Regional Water Board may consider permitting a proposed discharge under a site-specific waste discharge requirement order, or other administrative mechanism.

4. **Our agency operates a wastewater treatment facility that discharges to surface water and would like to enroll under this permit to use recycled water within our service area. The wastewater treatment facility will produce less discharge volume to surface water as a result. Does our agency need to obtain additional authorization?**
   Yes. Diversion of recycled water that would otherwise be discharged to a watercourse requires additional consideration to protect downstream and in-stream uses. Water Code section 1211 requires State Water Board approval before changing a surface water point of discharge.

General Order Coverage

1. **What can be permitted under the General Order?**
   Only treated municipal wastewater for nonpotable uses can be permitted with the General Order. The General Order establishes standard conditions for recycled water use and conditionally delegates authority to an administrator to issue Recycled Water Use Permits to recycled water users. Recycled water users are anyone proposing to use recycled water; this can be a public agency (a water system using recycled water for irrigation of local parks) or private users (an individual farmer using recycled water for crop irrigation, a private resident picking up recycled water for landscape irrigation, or a utility company using recycled water for cooling towers).

   The General Order provides regulatory coverage for certain uses of recycled water that are consistent with requirements of California Code of Regulations, Title 22. Some of the common uses of recycled water include irrigation of landscaping, athletic fields, crops, and certain industrial uses. Other uses not listed in California Code of

Regulations, Title 22 may be considered. Requirements for these uses will be set by the State Water Board and Regional Water Boards for protection of public health.

2. **What recycled water uses are not eligible for coverage under the General Order?**
   Use of recycled water for potable use, activities to replenish groundwater resources and activities to simply dispose of treated wastewater are not eligible for enrollment in the General Order.

3. **Our agency has a master reclamation permit that covers our recycled water production facility and several large industrial recycled water users. We would like to expand the extent of our recycled water program to cover landscape irrigation. Do we need to amend or rescind our existing master reclamation permit coverage?**
   It may be unnecessary to amend or rescind the existing master reclamation permit coverage. This General Order may be used to streamline the addition of new uses not currently covered under an existing recycled water permit. It is not intended to create duplicative requirements for use of recycled water under this order. Agencies with existing Regional Water Board permit coverage for recycled water use are highly encouraged to consult with their Regional Water Board contacts early in the process to make sure that the permit coverages do not overlap.

4. **Our agency has a linear utility construction project that spans multiple Regional Water Board boundaries. Can we submit a single application package?**
   Yes. A single application package can be submitted to the State Water Board Division of Drinking Water. State Water Board staff will coordinate the review and processing of the project with each Regional Water Board to make sure that the proposed application meets the requirements of the General Order, including compliance with each Regional Water Board's Water Quality Control Plan. The State Water Board will process and issue the Notice of Applicability. Monitoring reports prepared by the administrator for compliance with the General Order can also be submitted to the State Water Board Division of Drinking Water. The administrator is the party legally responsible for compliance with the General Order. See "Administrator's Role" described below for more information.

Application Process

1. **Who can apply for coverage under the General Order?**
   The General Order may be issued to recycled water producers, distributors, or a legal entity (such as a joint powers authority). A single user of recycled water can be permitted with the General Order (the user would fill the role of administrator). The application process is described in General Order Attachment A. Administrators may elect to issue Recycled Water Use Permits to users (as described below).

2. **How can I get coverage under the General Order?**
   There are two ways to get coverage under the General Order, and how you apply depends upon your status. If you will be an administrator (or the sole user), you would apply to the Regional Water Board. If you are a user and an administrator has been established, you apply to the administrator's program. If you are uncertain whether an administrator has been established, contact the wastewater treatment system operator for information on the

availability of recycled water. Administrators that are not wastewater treatment operators must coordinate with the wastewater treatment facility before submitting a Notice of Intent (NOI) to the Regional Water Board.

Administrator Application Process
An applicant submits an NOI (see directions in General Order Attachment A) to the Regional Water Board, and a Title 22 Engineering Report for the use of recycled water to the State Water Board Division of Drinking Water (DDW) field office.[3] Note that the Title 22 Engineering Report must be approved before the Regional Water Board can process the NOI. Allow approximately 90 days for Regional Water Board processing once the NOI is complete. The Regional Water Board will issue a Notice of Applicability (NOA) to the administrator to authorize the recycled water use and distribution program.

User Application Process
An administrator authorized to distribute recycled water will issue recycled water use permits to users. In this case, the administrator is the permitting agency rather than the Regional Water Board. If you are a user, submit your application to the administrator in accordance with their requirements.

3. Is there a fee? How much?
Yes. An annual fee is required; the first-year fee is paid with the NOI application package. The fee amount is based on the threat to water quality. In some circumstances, water recycling entities that are currently paying fees for coverage under an existing master reclamation permit proposing coverage under this General Order for a simple addition of a new use type (for example: adding only construction water program) may not need to pay additional fees. Contact your Regional Water Board representative to determine your fee amount. The water quality fee schedule is posted at http://www.waterboards.ca.gov/resources/fees/water_quality/

Division of Drinking Water fees are billed at an hourly rate directly to the water recycling entity. The Division of Drinking Water fee schedule is posted at http://www.waterboards.ca.gov/resources/fees/drinking_water/

4. Our agency recently submitted an updated Title 22 Engineering Report for our recycled water production facility. The same Title 22 Engineering Report for our production facility is being requested as a part of our submittal of our Water Recycling Program technical report. Do we need to resubmit a duplicate copy?
Contact your Regional Water Board representative to determine the scope of your Notice of Intent (NOI) submittal to enroll under the General Order. Regional Water Board staff has the discretion to require or waive some of the information in the NOI Water Recycling Program technical report, particularly if the Regional Water Board already received an identical submittal for another permitting activity.

[3] State Water Board Division of Drinking Water field offices are available at: http://www.waterboards.ca.gov/drinking_water/programs/documents/ddwem/DDWdistrictofficesmap.pdf

Administrator's Role

1. **What does an administrator do?**
   The administrator establishes and enforces rules for recycled water use and issues recycled water use permits to users. All recycled water use permits must be consistent with an approved Title 22 Engineering Report, the General Order, and the NOA. The administrator is responsible for paying the annual fee to the State Water Board, ensuring recycled water use is consistent with the requirements and that monitoring and reporting is completed on time. Water recycling administration requirements are described in the General Order.

2. **What if an administrator is also a sole user in the Recycled Water Program?**
   The administrator is then responsible to implement water recycling administration requirements applicable to users and administrators. An example of this responsibility can be as simple as instead of inspecting user sites subject to recycled water use permits, the administrator performs inspections of its own recycled water use areas.

Monitoring and Reporting

1. **Is monitoring of recycled water use required?**
   Yes. Monitoring and reporting to the Regional Water Board is required to demonstrate compliance with the General Order, the Title 22 Engineering Report and the NOA. The Regional Water Board will prepare a site-specific monitoring and reporting program based on site conditions.

2. **What other types of monitoring and reporting would be required?**
   The General Order includes a template Monitoring and Reporting Program (MRP) that can be modified by a Regional Water Board's executive officer or a State Water Board's executive director (or designee) pursuant to Water Code section 13267. These modified MRPs can be more or less than what is provided in the template MRP depending on the complexity of the proposed Recycled Water Program and any necessary compliance with the Regional Water Board's Water Quality Control Plan.

Applicable Plans, Policies and Regulations

1. **Where can I find recycled water-related statutes and regulations?**
   Title 17 and Title 22 regulations related to recycled water are posted at http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/Lawbook.shtml

2. **Where can I find the Regional Water Boards' water quality control plans (basin plans)?**
   Each Regional Water Board posts its water quality control plans on its website. To locate each Regional Water Board of jurisdiction, enter a project address or click on a Regional Water Board location at http://www.waterboards.ca.gov/waterboards_map.shtml, and search for "Basin Plan."

3. **Where can I find the State Water Board's Recycled Water Policy?**
   The State Water Board's Recycled Water Policy is posted at http://www.waterboards.ca.gov/water_issues/programs/water_recycling_policy/index.shtml

Constituents Associated with Recycled Water

1. **What constituents are associated with recycled water?**
   Constituents associated with recycled water that have the potential to degrade groundwater include salinity, nutrients, pathogens (represented by coliform bacteria), disinfection byproducts, and endocrine disruptors. The General Order addresses how recycled water use, if done in accordance with the requirements of the General Order, will not unreasonably affect beneficial uses or impair water quality.

2. **How are constituents of emerging concern (CECs) being addressed in the General Order?**
   The General Order acknowledges the presence of constituents of emerging concern in recycled water consistent with the State Water Board's Recycled Water Policy, which relies on the recommendations of a Science Advisory Panel. One of the Science Advisory Panel's charges is to provide recommendations on monitoring CECs for three reuse practices in which CECs may represent a potential threat to human health, including groundwater replenishment by surface application (surface spreading); groundwater replenishment by subsurface application (subsurface injection); and urban landscape irrigation.

   The General Order permits only nonpotable uses of recycled water (such as urban landscape irrigation) and does not require any CECs monitoring. The Science Advisory Panel concluded that, while human exposure to CECs can occur through incidental contact with and accidental consumption of recycled water from sprinkler heads, faucets, or hydrants, it does not warrant a monitoring program for CECs to protect public health.

   Recommendations of the Science Advisory Panel on monitoring strategies for CECs in recycled water is posted at:
   http://www.waterboards.ca.gov/water_issues/programs/water_recycling_policy/docs/cec_monitoring_rpt.pdf

3. **Is it safe to eat fruits or vegetables from crops irrigated with recycled water? How is this addressed in the General Order?**
   Use of recycled water for agricultural irrigation, including food crops, is addressed in the Uniform Statewide Recycling Criteria. In 2012, the California Department of Public Health convened an expert panel to consider whether recycled water produced under California's Uniform Statewide Recycling Criteria sufficiently protects public health for agricultural food crop irrigation. The report specifically addressed the risk of exposure and infection from waterborne pathogens, such as Cryptosporidium and E. coli, due to the irrigation of a wide variety of food crops using recycled water. The panel concluded that "current agricultural practices that are consistent with the (Water Recycling Criteria) do not measurably increase public health risk, and that modifying the standards to make them more restrictive will not measurably improve public health."

   The potential presence of human pathogens in recycled water and their uptake into plant tissue via the root system, leaf stoma, etc., were addressed as potential concerns. The Independent Advisory Panel finds there is evidence that plant uptake may occur under laboratory conditions with exposure to a high concentration of pathogens. However, it is

more likely that the pathogens attach to plant surfaces in such a way that processing sanitization or other intervention becomes less effective. This latter scenario is the probable mechanism of contamination associated with foodborne outbreaks referenced in the Independent Advisory Panel's report, none of which were associated with use of recycled water for irrigation.

The General Order requires use of recycled water for irrigation, including those for food crops, to meet the requirements of the Uniform Statewide Recycling Criteria. Recycled water, if used for agricultural irrigation, typically supplements other water supply sources such as surface water and groundwater, which results in plant exposures far lower than those tested under laboratory conditions.

Recommendations from the National Water Research Institute (NWRI) Independent Advisory Panel's report, titled "Review of California's Water Recycling Criteria for Agricultural Irrigation" is posted at: http://nwri-usa.org/cdph.ag.htm

4. **Endocrine disruptors such as perchlorate may be present in disinfected recycled water, absorbed by fruit-producing trees, and concentrated on the fruits. Does this General Order contain any requirements to address perchlorate in recycled water?** Recycled water uses proposed by an administrator's Recycled Water Program must meet the Uniform Statewide Recycling Criteria and any other standards set by the State or Regional Water Boards for protection of public health. The Uniform Statewide Recycling Criteria was reviewed by an expert panel to determine whether it is sufficiently protective of public health for agricultural food crop irrigation. Based on literature and monitoring data reviewed, recycled water is a relatively insignificant source of perchlorate based on type and volume of recycled water used for agricultural irrigation, and levels of perchlorate monitored in facilities that discharge to surface water.

   While there is no specific requirement addressing perchlorate in the General Order, it was considered in preparation of the General Order as documented in a staff memorandum addressing perchlorate occurrence in sources of agricultural water supplies. This memorandum is posted at: http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/requirements.shtml Based on literature and monitoring data reviewed, recycled water is a relatively insignificant source of perchlorate based on (1) type and volume of recycled water used for agricultural irrigation and (2) levels of perchlorate monitored in facilities that discharge to surface water (17 NPDES facilities out of 214 facilities, 12 out of 17 facilities are recycled water production facilities).

For more information on the General Order for Recycled Water, contact the Division of Drinking Water.

(This fact sheet was last updated January 22, 2016.)

EXHIBIT 13

TO: Recycled Water General Order Project File

DATE: July 25, 2014

SUBJECT: SUMMARY OF PERCHLORATE OCCURENCE IN SOURCES OF AGRICULTURAL WATER SUPPLIES

This memo summarizes information regarding perchlorate, its anthropogenic and natural sources, its occurrence in treated wastewater (recycled water), and its presence in surface and groundwater supply sources. Perchlorate is both a synthetic and a naturally occurring chemical that is soluble in water, mobile in groundwater, and persistent in groundwater. California regulates perchlorate in drinking water and established an MCL of 6 ug/L. There is currently no established federal MCL or agricultural water quality goal (published by the Food and Agriculture Organization of the United Nations) for perchlorate.

Review of recycled water use for agricultural irrigation indicates that the perchlorate originating from recycled water is a relatively insignificant source of perchlorate compared to other sources. This determination is summarized below:

1. Available data indicates the concentration of perchlorate in disinfected wastewater is nearly always less than the MCL.
2. Not all recycled water used for agricultural is disinfected. Some portion of recycled water used for agricultural use is either not disinfected, or is disinfected by means that do not result in perchlorate generation.
3. Recycled water makes up less than 1 percent of the agricultural water supply. In most cases, recycled water supplements the regular irrigation water supply.
4. Other sources of agricultural water supply contain perchlorate, often at concentrations higher than the recycled water perchlorate concentrations.
    - The Colorado River supplies 13 percent of the agricultural water in the state and it contains 5 to 9 ug/L perchlorate.
    - Groundwater in some areas of the state (especially Riverside, San Bernardino, and Los Angeles Counties) has been impacted with perchlorate. Typically, they are in areas near an industrial site that used perchlorate for an industrial purpose.
    - The volume of water exported through the Sacramento San Joaquin Delta (Delta) or locally pumped groundwater makes the contribution of perchlorate

from recycled water insignificant. If perchlorate is not present in either the water exported from the Delta or pumped from agricultural wells, the perchlorate in recycled water is significantly diluted. If perchlorate is present in either or both of the water supplies, the perchlorate contributed by recycled water is insignificant.

- Approximately 51 percent of agricultural water is delivered through the Sacramento-San Joaquin Delta. No perchlorate data is available for that water.
- Approximately 35 percent of agricultural water is delivered through agricultural production wells. No perchlorate data is available for that water.

5. Other sources of perchlorate may be contributing significant amounts of perchlorate to surface and groundwater supplies.

Additional discussion of the summary provided above is presented below.

1. Available data indicates the concentration of perchlorate in disinfected wastewater is nearly always less than the MCL.

Disinfection of Wastewater as a Perchlorate Source

Agricultural irrigation with recycled water is allowed under title 22. In general, higher levels of exposure to recycled water require higher levels of treatment and disinfection. Depending upon the crop irrigated, the recycled water may be undisinfected or meet specific disinfection criteria. Title 22 requires some recycled water to be disinfected. One way to disinfect recycled water is the use of sodium hypochlorite. However, sodium hypochlorite solutions may also contain perchlorate.[1] As a result, recycled water disinfected with sodium hypochlorite can add perchlorate to recycled water. (As will be discussed later in this memo, there are other sources of perchlorate in the environment.) To determine if perchlorate in recycled water is a significant source of perchlorate in the environment, State Water Board staff reviewed wastewater treatment system effluent data that is available electronically. The CIWQS database includes analytical data that has been electronically uploaded by major NPDES dischargers. (Major NPDES dischargers consist of NPDES permitted municipal wastewater facilities with flows greater than 1 MGD.)

CIWQS Review of Wastewater Treatment Plants with Perchlorate Data

Of the 214 major NPDES facilities listed in CIWQS (flows greater than 1 MGD), 17 facilities monitor for perchlorate concentrations in their effluent, 12 of the 17 facilities are water recycling facilities. A review of from January 2011 – July 2014 indicates perchlorate is sometimes present. When measureable perchlorate is present, it is generally below 2 ug/L. One facility reported a perchlorate concentration of 10 ug/L in a single sample event. (That was the only perchlorate data available for that discharger.)

[1] Perchlorate may form in hypochlorite solutions during manufacturing and storage. <http://www.forceflow.com/hypochlorite/Perchlorate_in_sodium_Hypo.pdf>

2. Not all recycled water used for agricultural is disinfected. Some portion of recycled water used for agricultural use is either not disinfected, or is disinfected by means that do not result in perchlorate generation.

Recycled water makes up a very small percentage (less than one percent) of the total water used for agriculture in California. Of that recycled water use, only some portion is disinfected with sodium hypochlorite, other disinfection methods are ultraviolet (UV) light, and chlorine gas (which is unlikely to create perchlorate in the treated wastewater). Furthermore, some portion of recycled water used for agricultural irrigation uses undisinfected secondary recycled water for irrigation of orchards where the recycled water does not come into contact with the edible portion of the crop, non-food bearing trees, fodder and fiber crops for animals not producing milk for human consumption, seed crops not eaten by humans, food crops that must undergo commercial pathogen-destroying processing, and ornamental nursery stock and sod farms. Disinfection derived perchlorate is not present in undisinfected secondary recycled water.

3. Recycled water makes up less than 1 percent of the agricultural water supply. In most cases, recycled water supplements the regular irrigation water supply.

California agricultural uses approximately 27 million acre feet of water per year. Approximately 51 percent of the water supply comes from the Sacramento – San Joaquin Delta and 35 percent comes from groundwater wells; the Colorado River supplies approximately 13 percent. Approximately 1 percent of irrigation water is supplied from recycled water. Table 1 presents estimates of sources of water used for agricultural in California.

4. Other sources of agricultural water supply contain perchlorate, often at concentrations higher than the recycled water perchlorate concentrations.

<u>Perchlorate in Surface Water Supply Sources</u>
There is no available data on perchlorate monitoring for the Sacramento-San Joaquin Delta water. Similarly, there is no perchlorate monitoring data for agricultural wells. Colorado River water sampling has shown perchlorate concentrations range from 5 – 9 ug/L. Table 2 presents a summary of perchlorate concentrations in various sources.

<u>Perchlorate in Groundwater Supply Sources</u>
Thousands of active and standby public water supply wells were sampled by the California Department of Public Health (CDPH) for perchlorate. As of February 2012, 312 active and standby public water supply wells out of 10,952 sampled had perchlorate concentrations above the MCL. Peak concentrations were reported as high as 108 ug/L in Los Angeles County, 68 ug/L in Riverside County, and 94 ug/L in San Bernardino County. CDPH maintains an updated summary of active and standby sources with perchlorate detections; Table 3 presents a summary of the 2010 – 2013 perchlorate data.

Although agricultural wells were not sampled in the CDPH investigation, it is reasonable to assume agricultural wells have the potential to contain perchlorate in areas where perchlorate containing fertilizer (explained in Item 5 below) was or is used. Agricultural wells are typically more vulnerable to contamination considering that agricultural wells may draw from shallower zones, the wells may be older and have deteriorated casings, or the wells may have been

constructed in a way that does not provide an effective sanitary seal (e.g. cable tool wells, well points, open borehole completions).

5. Other sources of perchlorate may be contributing significant amounts of perchlorate to surface and groundwater supplies.

Anthropogenic and Natural Sources of Perchorate

Common anthropogenic sources of perchlorate include perchlorate salts used in industrial or military applications, solid rocket fuels, explosives, fertilizers, automotive air bag inflators, electroplating, aluminum refining, fireworks, matches, road flares, and production of paints and enamels.

Perchlorate is naturally present in some fertilizers that have been used in the United States since the early 20th century. Chilean nitrate fertilizer containing naturally-occurring perchlorate has been widely used in American agriculture. Between 1923 and 1998, the reported usage of Chilean fertilizer in California was 477,061 metric tons. Though the quantities used today are smaller than the amounts applied earlier in the century, the use of Chilean nitrate fertilizer in California remains substantial. According to the 2000 United States Census, more than 6,600 tons of Chilean nitrate fertilizer was imported to California that year. ("Perchlorate Basics" 2010, Perchlorate Information Bureau. http://perchlorateinformationbureau.org/perchlorate-basics)

The USGS recently published a study reporting natural levels of perchlorate in desert soil, plants, and atmospheric materials. The research found shallow soils in the USGS Amargosa Desert Research Site in Nevada contained a high level of perchlorate, about 10 – 20 grams per hectare (0.1 – 0.3 ounce per acre) in the top one foot of soil. The equivalent amount, if flushed to groundwater, would be sufficient to result in a quarter million gallons of water per acre exceeding the California MCL. ("Natural Perchlorate Levels in a Desert Ecosystem." 3 April 2014. USGS Newsroom. http://www.usgs.gov/newsroom/article.asp?ID=3859#.U8_Dy_ldWhY)

Enc: Table Sheet and Notes Page

TABLE 1: ESTIMATE OF SOURCES OF WATER USED FOR AGRICULTURAL IN CALIFORNIA

Source	Flow	Proportion of Total Water Supply	Notes
Sacramento - San Joaquin Delta	14,090 TAF	50.8%	*(See Note 7)*
Colorado River	3,716 TAF	13.4%	*(See Note 6)*
Groundwater wells	9,660 TAF	34.9%	*(See Note 8)*
Municipal recycled water	245 TAF	0.9%	*(See Note 4)*
Total	27,711 TAF		*(See Note 9)*

TAF denotes thousands of acre feet.

TABLE 2: SUMMARY OF PERCHLORATE CONCENTRATION IN VARIOUS SOURCES

Source	Concentration	Notes
Sacramento - San Joaquin Delta	Not documented	*(See Note 1)*
Colorado River	5 - 9 ug/L	*(See Note 2)*
Municipal supply wells	up to 108 ug/L	*(See Note 3 & Table 3)*
Municipal wastewater	0 - 10 ug/L	*(See Note 5)*

TABLE 3: ACTIVE AND STANDBY GROUNDWATER SUPPLY SOURCES WITH PERCHLORATE DETECTIONS (2010 - 2013) (SEE NOTE 3)

	Peak detection at or above 4 ug/L		Peak detection above 6 ug/L		Peak Concentration
County	No. of Sources	No. of Systems	No. of Sources	No. of Systems	(ug/L)
Contra Costa	1	1	1	1	7.9
Fresno	1	1	-	-	4.5
Kern	2	2	1	1	14
Los Angeles	98	31	68	21	108
Monterey	1	1	-	-	4.8
Orange	11	7	3	2	9
Riverside	49	8	38	8	68
Sacramento	2	1	2	1	13
San Bernardino	57	19	37	16	94
San Diego	10	2	8	2	9.9
Santa Barbara	1	1	-	-	4.6
Santa Clara	5	4	3	3	10
Sutter	3	3	1	1	10
Tulare	6	3	5	3	20
Ventura	1	1	-	-	5.2
Total	248	85	167	59	-

NOTES:

1) There is currently no monitoring of Delta waters for perchlorate. http://www.waterrights.ca.gov/baydelta/docs/exhibits/append2/DK-02.pdf

2) Groundwater Information Sheet Perchlorate. State Water Resources Control Board. February 2012. http://www.waterboards.ca.gov/gama/docs/coc_perchlorate.pdf

3) Perchlorate in Drinking Water . California Department of Public Health. February 2014. http://www.cdph.ca.gov/certlic/drinkingwater/Pages/perchlorate.aspx

4) 2009 Municipal Wastewater Recycling Survey. State Water Resources Control Board. Data is for agricultural irrigation. http://www.waterboards.ca.gov/water_issues/programs/grants_loans/water_recycling/munirec.shtml

5) State Water Resources Control Board CIWQS data for NPDES Majors facilities (flow > 1 MGD) monitoring. Out of 214 facilities, 17 facilities have effluent monitoring for perchlorate. 12 out of 17 facilities are water recycling facilities. Discoverer Plus, accessed July 24, 2014.

6) Pacific Institute. Water to Supply the Land: Irrigated Agriculture in the Colorado River Basin. May 2013. pp 46-51 http://pacinst.org//wp-content/uploads/sites/21/2013/05/pacinst-crb-ag.pdf. Data is for 2005. USBR records of average annual consumptive use for years 2002-2005.

7) Lund, Jay et al. Envisioning Futures for the Sacramento-San Joaquin Delta. February 2007. Chapter 6. http://www.ppic.org/content/pubs/report/R_207JLChapter6R.pdf. Table 6.1 Estimated Average Consumptive Uses of Delta and Delta Tributary Waters, 1995-2005 (taf/year). Data is for total diversions of agricultural demand area.

8) Kenny, Joan, et al. USGS Circular 1344 Estimated Use of Water in the United States in 2005. Table 7 Irrigation water withdrawals, 2005. Table entry for California. http://pubs.usgs.gov/circ/1344/pdf/c1344.pdf

9) Calculated total water supply is within 3% of USGS Circular 1344 Estimated Use of Water in the United States in 2005. Table 7 Irrigation water withdrawals, total 27,300 TAF. http://pubs.usgs.gov/circ/1344/pdf/c1344.pdf

EXHIBIT 14

MEMORANDUM OF AGREEMENT BETWEEN
THE DEPARTMENT OF HEALTH SERVICES
AND
THE STATE WATER RESOURCES CONTROL BOARD
ON USE OF RECLAIMED WATER

This Memorandum of Agreement (hereafter MOA) is made between the Department of Health Services (hereafter Department) and the State Water Resources Control Board (hereafter SWRCB) on behalf of itself and the nine California Regional Water Quality Control Boards (hereafter RWQCBs). This MOA sets forth principles, procedures, and agreements to which these agencies commit themselves relative to use of reclaimed water in California. It is effective upon the date that it is executed by both parties.

I. PURPOSE AND SCOPE OF MOA

Water reclamation involves several activities that have potential impacts on public health. The primary activities are the introduction of pollutants into the wastewater collection system, wastewater treatment, storage and distribution of reclaimed water, and the use of the reclaimed water. The planning, design, construction, and operation of the various facilities associated with these activities all require oversight by regulatory agencies to ensure protection of public health.

This MOA is intended to assure that the respective authority of the Department, the SWRCB, and the RWQCBs relative to use of reclaimed water will be exercised in a coordinated and cohesive manner designed to eliminate overlap of activities, duplication of effort, gaps in regulation, and inconsistency of action. To that end, this establishes basic principles relative to activities of the agencies hereto and the RWQCBs, clarifies primary areas of responsibility and authority between these agencies, and provides for methods and mechanisms necessary to assure ongoing, continuous future coordination of activities relative to use of reclaimed water in this State.

The MOA is intended to serve as an umbrella agreement between the agencies hereto. It will be supplemented, as appropriate, by addenda which will reflect any additional agreements, commitments and understandings arrived at by the agencies hereto. This MOA replaces the previous MOA on use of reclaimed water executed on 5 December 1988.

II. GENERAL BACKGROUND

A. Basic Authorities and Responsibilities

In order to supplement existing surface and ground water supplies to help meet water needs in the State, it is State policy that use of reclaimed water in the State be promoted to the maximum extent (California Water Code, Sections 13510-13512). One of the primary conditions on the use of reclaimed water is protection of public health (Water Code Sections 13521, 13522, 13550(a)(3)).

The Department is the primary State agency responsible for protection of public health and the regulation of drinking water. The Legislature has defined several specific regulatory responsibilities of the Department related directly or indirectly to water reclamation activities including: establishment of statewide water reclamation criteriaadvising RWQCBs in the drafting of water reclamation requirements (permits); review and approval of certain proposed water reclamation projects; abatement of contamination resulting from use of reclaimed water where public health is seriously threatened; and control of cross connections between potable and nonpotabie water systems.

The SWRCB and the RWQCBs are the primary State agencies charged with the protection, coordination, and control of water quality and the assignment of water rights in the State. Specific regulatory responsibilities affecting water reclamation include approval of pollutant source control programs for wastewater collection systems, issuance and enforcement of water reclamation requirements to producers and users of reclaimed water, definition of beneficial uses of surface and ground water bodies through the establishment of water quality control plans, regulation of operators of wastewater and water reclamation treatment plants, and water right determinations regarding water reclamation.

To assure protection of public health where reclaimed water use is involved, the Department has been statutorily directed to establish uniform statewide reclamation criteria for the various uses of reclaimed water (Water Code Section 13521). The Department has promulgated regulatory criteria which are currently set forth in Title 22, Division 4, Section 60301 et seq., California Code of Regulations. The Department's regulatory criteria include specified approved uses of reclaimed water, numerical limitations and requirements, treatment method requirements and performance standards. The Department's regulations allow use of alternative methods of treatment, in some cases, so long as the alternative methods used are determined by the Department to assure equivalent treatment and reliability.

B. Water Reclamation Requirements and Reports

All persons who reclaim or propose to reclaim water, or who use or propose to use reclaimed water, must file a report with the appropriate RWQCB (Water Code Section 13522.5). If a RWQCB determines that it is necessary to protect public health, safety, or welfare, it may prescribe water reclamation requirements where reclaimed water is used or proposed to be used (Water Code Section **13523**). Where regulatory criteria have been adopted, no person may either reclaim water or use reclaimed water until the appropriate RWQCB has either issued reclamation requirements or waived the necessity for such requirements (Water Code Section 13524). In the process of issuing reclamation requirements, the RWQCBs must consult with and consider recommendations of the Department (Water Code Section 13523). Any reclamation requirements which are issued by the RWQCBs, whether applicable to the reclaimer or to the user of reclaimed water, must be in conformance with any regulatoryreclamation criteria adopted by the

Department. Water reclamation requirements for a proposed use of reclaimed water that is not specifically addressed in the Title 22 water reclamation criteria adopted by the Department are considered on a case-by-case basis.

The RWQCBs have the option of issuing a master reclamation permit in lieu of individual water reclamation requirements for a project involving multiple users. Such permits would combine the waste discharge requirements pursuant to Water Code Sections 13260 et seq. and water reclamation requirements. A master permit may be issued to a supplier or distributor, or both, of reclaimed water. The procedures for adoption by the RWQCBs are the same as for water reclamation requirements and include the same consultation with the Department (Water Code Section 13523.1). Except upon written request from a RWQCB, the reporting requirement in Section 13522.5 is waived for users supplied with reclaimed water from a supplier or distributor operating under a master permit (Water Code Section 13522.5). However, other reporting and plan review requirements, such as those specified in the Title 22 reclamation criteria, may be included as requirements in the master permit. In addition the RWQCBs have the option of issuing general waste discharge requirements or general water reclamation requirements, under which all producers of reclaimed water may apply to be covered, in lieu of individual orders.

Water Code Section 13554.2(e) requires the Department to review and approve proposed water reclamation projects (within specified time frames) that are submitted to the Department by producers or distributors of reclaimed water for review. The Department may delegate some or all of its responsibilities, with respect to review and approval of a proposed project, to a local health department with the concurrence of the project proponent (Water Code Section 13554.2(c)). The reclaimed water producer or distributor submitting the proposed project for review must reimburse the Department for its cost of conducting the review and issuing the approval or denial (Water Code Section 13554.2(a)).

Where reclaimed water use is involved or proposed, the RWQCBs have the authority to require construction reports and such other reports as may be necessary to assure protection of both public health and water quality (Water Code Section 13523). Additional engineering, construction, and operational reports are specified in the Title 22 criteria adopted by the Department.

C. Regulatory Enforcement

Where use of reclaimed water is involved, the RWQCBs have the exclusive authority to enforce water reclamation requirements. In extreme cases involving serious public health threats, the Department may take steps to abate any contamination which may result from use of reclaimed water (Water Code Section 13522). The RWQCBs may undertake various enforcement actions, both of a civil nature and relative to criminal sanctions, for failure to file necessary reports, for reclamation or use of reclaimed water without reclamation

requirements, or for violation of any reclamation requirements imposed by a RWQCB (Water Code Sections 13522, 13522.7, and 13525).

In addition to the authority vested in the SWRCB, the RWQCBs, and the Department relative to the use of reclaimed water, various local health agencies have an independent and autonomous role and authority to impose additional requirements and take enforcement actions with respect to water reclamation pursuant to local ordinances.

D. Cross Connection Control

The Department has responsibility for protection of potable water systems through cross connection control and backflow prevention. (Health and Safety Code Division 5, Part 1, Chapter 7.9, Sections 4049.50 et seq.; California Code of Regulations, Title 17, Division 1, Chapter 5, Group 4, Article 2, Sections 7601 et seq.). The Department has specified the backflow protection measures required at sites where reclaimed water is used.

E. Source Control

The federal Clean Water Act mandates municipal wastewater dischargers of 5 MGD or more into surface waters have an industrial pretreatment program (Clean Water Act, Sections 301 and 307). The purpose of this program is to control the input of constituents into sewer systems that could be harmful to wastewater treatment processes, treatment plant personnel, or the ability of a plant to meet effluent limitations. These requirements are implemented through the National Pollutant Discharge Elimination System (NPDES) permits issued by RWQCBs. Annual reports on the pretreatment programs submitted by the dischargers are reviewed by the RWQCBs. In addition, RWQCBs conduct inspections periodically to monitor these programs.

In the case of most water reclamation projects, all of the constituents of concern for public health protection are covered by current pretreatment programs. There is the potential that for certain types of reuse, particularly indirect potable reuse, some constituents would not come under the authority of the federal statutes to control through a pretreatment program. However, RWQCBs have the authority to include additional pretreatment program requirements or broader source control requirements in permits. Once such requirements are a part of a permit, the wastewater agency would be obligated to comply with the permit and the RWQCB would have authority to enforce the requirement.

F. Potable Water Supply Source Control

Planned indirect potable reuse of reclaimed water is commonly practiced in California through artificial ground water recharge with reclaimed water. Furthermore, indirect potable reuse is being proposed through the introduction of reclaimed water into a water supply reservoir that would serve as a raw water supply for a potable water system. The

Department has the responsibility to identify when and under what conditions a raw water supply is suitable for potable purposes.

G. Operator Certification

The qualifications of operators of wastewater treatment plants are determined by the SWRCB (Water Code Section 13627; California Code of Regulations Title 23, Chapter 26, Sections 3670 et seq.) Where water reclamation is involved, the SWRCB may require operators to be certified wastewater treatment plant operators. The water reclamation criteria promulgated by the Department states that operators of water reclamation plants shall meet the requirements for wastewater treatment plant operators specified by the SWRCB (California Code of Regulations, Section 60325).

H. Water Rights

Under certain conditions the use of potable water for nonpotable purposes is a waste or unreasonable use of water if reclaimed water is available (Water Code Sections 13550 et seq.). It is the responsibility of the SWRCB to make determinations under this provision. The SWRCB does not as a matter of course make this determination; such determination typically occurs in an adversarial proceeding after a complaint is filed. One of the conditions of the determination is that there is concurrence with the Department that the use of reclaimed water will not be detrimental to public health.

Prior to making any change in the point of discharge, place of use or purpose of use of treated wastewater, the owner of any wastewater treatment plant must obtain approval of the SWRCB (Water Code Sections 1210-1212). The Division of Water Rights of the SWRCB reviews and acts on such changes pursuant to the provisions of Section 1700 et seq. of the California Water Code. If a change in discharge or use of treated wastewater would occur due to a water reclamation project undertaken in response to a discharge restriction or other action by a RWQCB exercising its regulatory authority under Division 7 (commencing with Section 13000) of the Water Code, prior approval under Sections 1210 1212 is not required.

III. GENERAL PRINCIPLES

The general principles hereby agreed to by the Department, the SWRCB, and the RWQCBs are as follows:

A. All requests for water reclamation requirements submitted to a RWQCB pursuant to Section 13522.5 shall be considered to be a request for review by the Department pursuant to Section 13554.2, since Departmental review and recommendations are required by Section 13523.

B. Wherever feasible, the Department shall use the issuance of water reclamation requirements by a RWQCB as the preferred method of granting Departmental approval to a proposed project to avoid the issuance of separate project approvals by the Department.

C. Reclamation requirements issued by the RWQCBs will impose all applicable statewide reclamation criteria adopted by the Department and set forth in Title 22 regulations.

D. The Department will identify in its recommendations to a RWQCB with respect to proposed water reclamation requirements any conditions upon which its approval of a proposed project is based. The RWQCB staff will incorporate any "conditions of approval" submitted as part of the Department's recommendations into the water reclamation requirements proposed for adoption by the RWQCB.

E. Each agency hereto, when evaluating policies and procedures of its programs that affect water reclamation, shall consult with the other agency before adopting new policies or procedures.

F. Each agency hereto shall, to the maximum extent compatible with fulfillment of its primary responsibility to protect and preserve public health and water quality, promote and facilitate use of reclaimed water in this State.

G. As the primary enforcement agencies, the RWQCBs will enforce all aspects of the water reclamation requirements including the Title 22 regulatory requirements. The Department will provide technical assistance to the RWQCBs in carrying out the enforcement program. Where a public water system is involved in the supplying or distribution of the reclaimed water, the Department will use its enforcement authority over public water systems (such as cross connection control) to assist the RWQCBs in their enforcement efforts.

IV. PROGRAM PROVISIONS AND COMMITMENTS

To assure fulfillment of the purposes and principles set forth in this MOA, the agencies hereto commit themselves to the following programmatic approaches and procedures:

A. The RWQCBs will submit copies of proposed project reports or proposals to use reclaimed water as they are received rather than waiting until draft water reclamation permit requirements are completed in order to allow adequate time for review and, if necessary, preliminary discussion between the agencies.

B. The Department agrees to review and respond to water reclamation proposals and proposed water reclamation requirements within 30 days of receiving such referrals from the RWQCB. Should the Department determine that the project report is incomplete [per Water Code Section 13554.2(e)], it will immediately inform the RWQCB and indicate the additional information needed in order to complete the review of the proposed project.

C. In the event a recommendation of the Department is deemed by the RWQCB staff to be inappropriate for inclusion into water reclamation requirements, it will advise the appropriate District Office of the Department. The two agencies agree to meet and try to resolve any differences.

D. When requested by the Department, the RWQCB staff will incorporate a condition into a proposed master permit requiring the producer or distributor of the reclaimed water to submit plans, specifications, reports, or other specified material, to the Department for review and approval for specified new uses or new use areas that are added subsequent to the issuance of the master permit.

E. The Department will incorporate into any local delegation a requirement that the local agency abide by the terms and conditions of this MOA in the same manner as the Department.

F. When deemed necessary by the RWQCB, the Department will attend any RWQCB meeting or hearing to explain or defend any of the Department's conditions of approval or recommendations.

G. The RWQCBs will defer to the Department with respect to any questions involving interpretation of any Title 22 criteria.

H. RWQCB staffs will not waive reclamation requirements nor propose waiver of reclamation requirements for any proposed use of reclaimed water without consultation with the Department.

I. The agencies will work jointly to develop a definitive statement of policy and appropriate guidelines regarding the applicability of the ground water recharge regulations to various reclamation or wastewater disposal facilities.

J. The agencies agree to keep each other informed of any actions relating to specific projects and will send copies of all correspondence with project proponents or others that relate to a specific project to the other agency.

K. The Department agrees to try to coordinate its efforts with those of local health departments in order to foster a closer working relationship with local agencies and to reduce any potential conflicts for the RWQCBs.

L. In recognition of budget and staff limitations, the agencies hereto may be unable to fulfill all of the tasks outlined herein and, therefore, agree to commit to setting priorities that assure public health protection.

M. The RWQCBs will expeditiously notify the Department of all significant violations of reclamation requirements or improper reclamation uses within their jurisdictions. The Department will expeditiously notify the appropriate RWQCB of improper reclamation uses or violation of reclamation requirements which become known to the Department.

V. DISPUTE AND CONFLICT RESOLUTION

It is the desire of the agencies hereto to establish a speedy, efficient, informal method for resolution of interagency disputes, problems or conflicts. To that end, except as otherwise provided in this MOA, and to the extent not inconsistent with any formal administrativeappeals which may be pending, the agencies agree that:

A. Any concerns, issues or disputes, arising between the RWQCB staffs and the Department that cannot be resolved by meetings and discussions between the RWQCB Executive Officer and the Department's District Engineer will be brought to the attention of the Executive Director of the SWRCB. The Executive Director will attempt to resolve the matter to the satisfaction of both parties and will, if necessary, meet and confer with the Chief of the Department's Division of Drinking Water and Environmental Management.

B. Nothing contained herein shall be construed to deprive the Department of formal appeal rights relative to any alleged RWQCB action or inaction. In the event of such an appeal, the SWRCB will expedite any review process.

VI. MODIFICATION AND PERIODIC REVIEW

This MOA may be modified in writing at any time by mutual agreement of the agencies hereto. Proposed modifications may be suggested by any agency hereto at any time.

The agencies hereto will meet periodically, not less than once each year, to discuss the actions of each agency relative to this agreement, to devise and agree to appropriate activities for the forthcoming fiscal year, and to consider additional actions and activities which each agency can take to better coordinate their activities and further promote use of reclaimed water in the State.

Director
Department of Health Services

Date: 30 Jan 96

Executive Director
State Water Resources Control Board

Date: 25 Jan 96