Is Environmental Antibiotic Resistance a Fake Issue?

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Biosolids Workshop: Linking Industry and Research
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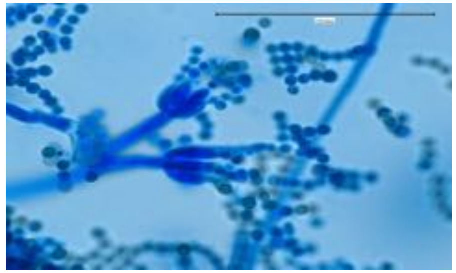
BACKGROUND

Antibiotics:

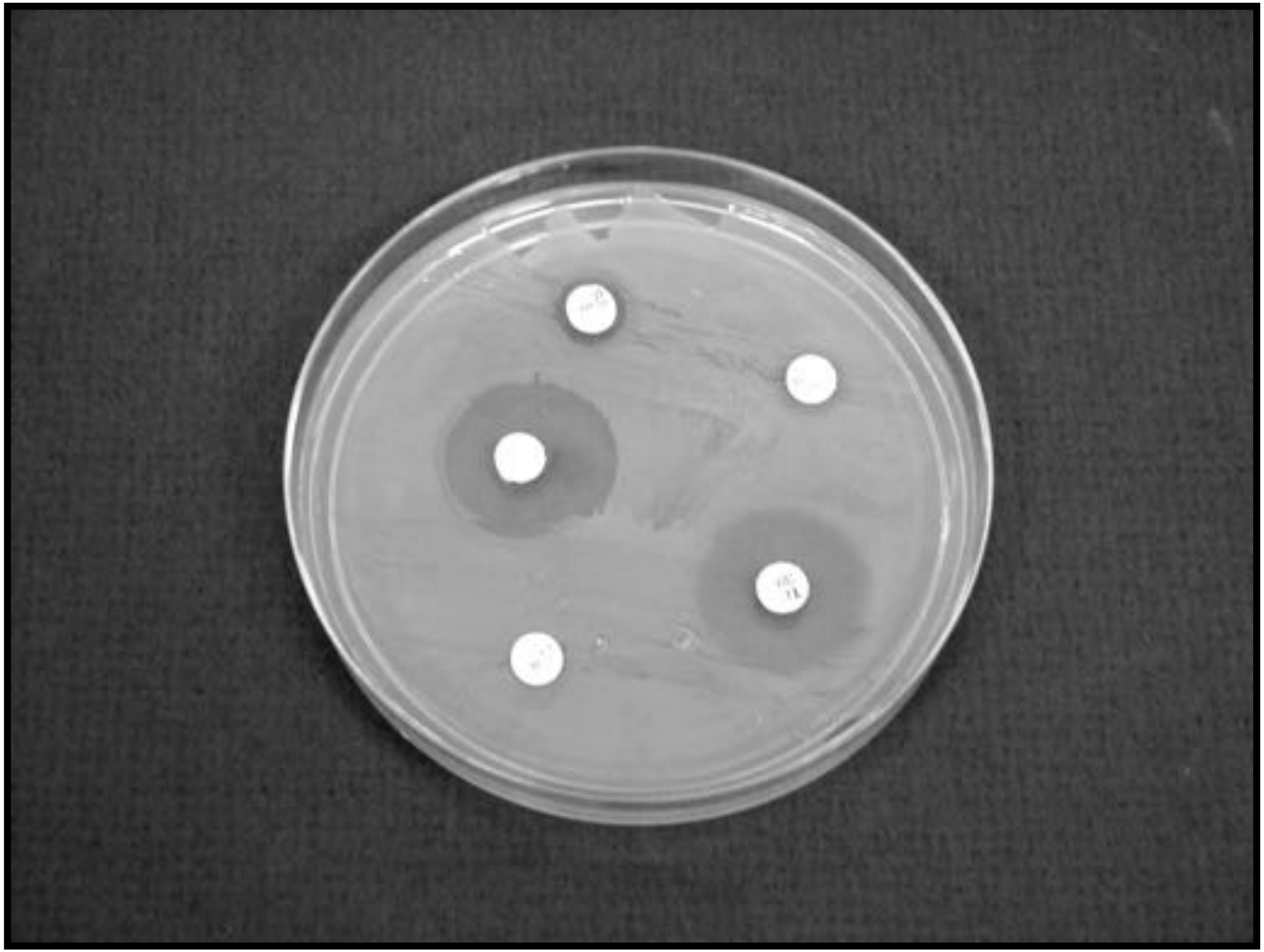
- Natural compounds produced by soil microorganisms that kill or inhibit the growth of other competing microorganisms



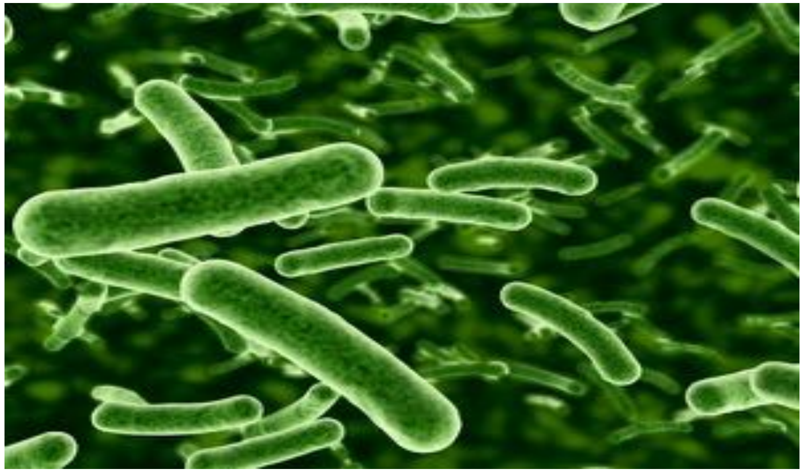
History:

- 1929 Alexander Fleming discovers penicillin isolated from soil fungus *Penicillium*
- 1943 Selman Waksman discovers streptomycin isolated from the soil actinomycete *Streptomyces*

Zone of inhibition of bacterial growth on a spread plate



BACKGROUND: The Concern



- The more an antibiotic is used the greater the likelihood of antibiotic resistant strains

- **The more the antibiotic is used to fight infectious disease, the less effective it becomes**

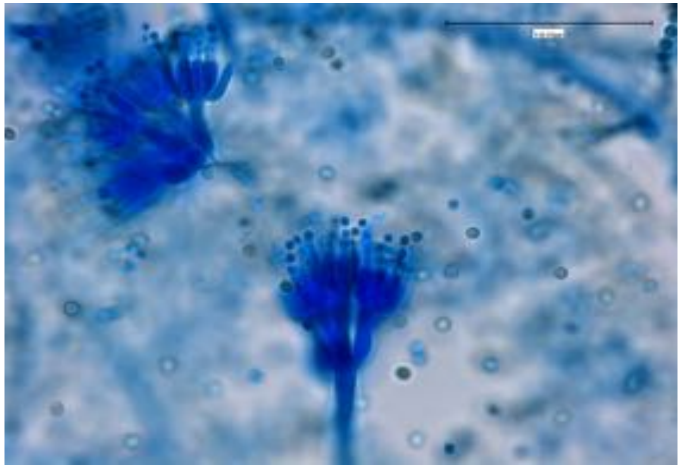
- Of particular concern, are bacteria resistant to multiple antibiotics

  e.g. Methicillin-resistant

  Staphylococcus aureus (MRSA)

MICROBIAL PRODUCTION OF ANTIBIOTICS

- Vast majority of antibiotics synthesized by soil microorganisms
  - Penicillin effective on Gm +ve bacteria
  - Polymixin effective on Gm –ve
  - Chloramphenicol effective against Gm +ve and -ve
- Antibiotics produced by bacteria, actinomycetes and fungi
- Utilized as major form of self-defense against other indigenous soil microbes



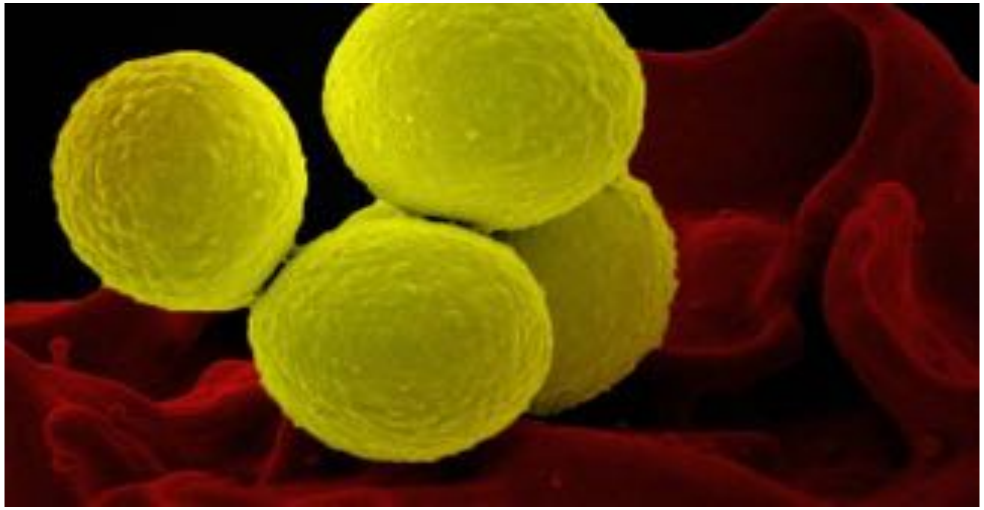
SOURCE OF ANTIBIOTICS
ARBs and ARGs

Anthropogenic:

- Sewage effluents and biosolids
- CAFO effluent and animal manures
- Hospital wastes discharged into sewers

Natural:

- Soils
- Water



ENVIRONMENTAL ANTIBIOTIC RESISTANCE

- New term introduced in 2013
- Caused by anthropogenic activity
- Wastewater treatment plants blamed for increasing "environmental antibiotic resistance"

Rizzo et al., 2013:
"Urban Wastewater Treatment Plants as Hotspots for Antibiotic Resistant Bacteria and Genes Spread into the Environment: A Review"

IS THIS TRUE: a fresh perspective?

SOIL BACTERIA

Culturable bacteria = $10^8 – 10^{10}$ per gram soil
Total bacteria = $10^{10} – 10^{12}$ per gram soil

One acre furrow slice $\simeq 2 \times 10^6$ lbs soil
(1 acre to depth of 6”)

= 9×10^8 grams soil

Assume 10^9 bacteria per gram soil

Therefore, $10^9 \times (9 \times 10^8)$ bacteria per acre furrow slice
$\simeq 10^{18}$

ANTIBIOTIC RESISTANT BACTERIA (ARB)

Naturally occurring in soil for 3 billion years
(Gaze et al., 2013)

Present in all soils even pristine soils

For any given antibiotic there are $10^6 - 10^7$ culturable ARB

Therefore, for 1 acre furrow slice we have:

$9 \times 10^8 \times 5 \times 10^6 \simeq 4.5 \times 10^{15}$ ARB

Table 1. Documented detectable levels of ARB and ARG in the environment.

Sample Type	ARB (CFU/g or CFU/ml)	Ref	ARG (# gene copies/g or ml)	Ref
Soil	$10^6 – 10^7$	Brooks et al., 2007	$10^8 – 10^{9*}$	See text
Class B Biosolids	$10^4 – 10^9$ 10^7	Munir et al., 2011 McCall et al., 2016	10^6 - 10^9	Munir et al., 2011
Treated Municipal Wastewater effluent	$10^1 – 10^3$	Gao et al., 2012	$10^4 – 10^7$	Munir et al., 2011
Poultry Manure	10^5 - 10^8	Brooks et al., 2010	$10^6 – 10^{11}$	Brooks et al., 2016 McKinney et al., 2010
Bovine Manure	10^5 - 10^8	Yang et al., 2010	$10^3 – 10^{10}$	Yang et al., 2010 Munir et al., 2011
Swine Manure	10^5 - 10^7	Sengelov et al., 2003	$10^4 – 10^9$	Brooks et al., 2014

IMPACT OF LAND APPLICATION OF BIOSOLIDS

Assume 2 metric tons biosolids applied per acre

2 metric tons = 2×10^6 g biosolids

From Table 1, geometric mean value of ARB/g

biosolids = 4.6 x = 10^6

Therefore, $4.6 \times 10^6 \times 2 \times 10^6$ ARB added

= 9.2×10^{12} additional ARB applied

% INCREASE DUE TO LAND APPLICATION

$$\% \, Increase = \frac{additional \ ARBs}{intrinsic \ soil \ ARBs}$$

$$\% \, Increase = \frac{9.2 \ x \ 10^{12}}{2.9 \ x \ 10^{15}}$$

$$\% \, Increase = 0.32\%$$

Table 2. Impact of land application of Class B biosolids, effluent and manure on ARB and ARG in soil using Table 1 as a reference.

Sample	ARB (CFU per acre furrow slice)	ARG (gene copies per acre furrow slice)
Soil	4.5×10^{15}	2.9×10^{17}
Biosolids applied, 2 Tons Per Acre	9.2×10^{12}	6.3×10^{13}
1 Acre Foot Effluent	1.2×10^{11}	3.9×10^{14}
2 Tons Per Acre Solid Manure	4.3×10^{12}	2.9×10^{13}

Table 3. Hypothetical percent increases of ARB and ARG in soil due to land application.

Material Applied	ARB (% increase)	ARG (% increase)
Biosolids	0.32	0.02
Effluent	0.0043	0.14
Manure	0.15	0.01

Table 4. Concentrations of pathogens measured in soil, Class B biosolids and treated wastewater.

A) Soil

Indigenous Pathogens	Ref	Number (per gram soil)	Number (per acre furrow slice)
Bacillus anthracis	Ganz et al., 2014	10^4	$\simeq 10^{13}$
Clostridium perfringens	Konishi et al., 1981	900	$\simeq 10^9$

B) Class B Biosolids

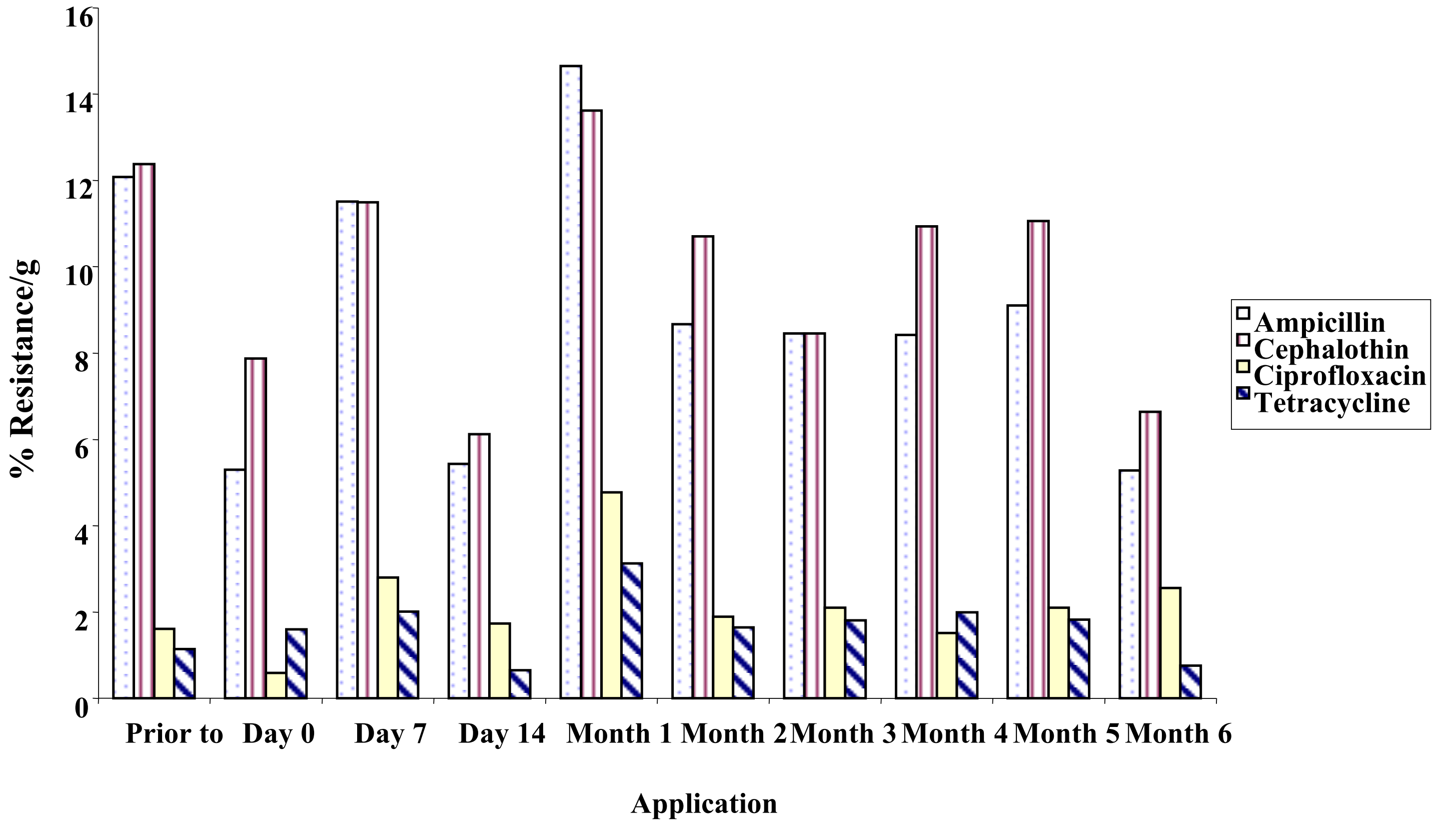
Pathogens	Number (per gram)	Number (per 2 tons biosolid)
Thermotolerant (fecal) coliforms (including *E. coli*)	10^6	10^{12}
Fecal *Streptococcus*	10^6	9×10^{12}
Salmonella	40	4×10^7

Pepper et al., 2010

BIGGEST ENVIRONMENTAL CONCERN

Animal manures particularly swine manure

- Publications show that ARG levels increase following land applications
- Decrease to background levels within 2 months



Percent antibiotic resistant bacteria in land applied biosolids

IMPACT OF SEWAGE EFFLUENTS AND CLASS B BIOSOLIDS ON PATHOGENIC ARBs IN SOIL

- Minimal ARBs added relative to what is already in soil
- Number of enteric pathogens (e.g. *E.coli*) introduced via effluent and biosolids is less than pathogens indigenous to soil (e.g. *Bacillus anthracis* or *Clostridium perfringens*)
- Enteric pathogens and ARBs introduced into soil normally die-off quickly
- When *E.coli* adapt to soil environment, pathogenicity lost
- Horizontal gene transfer in soil limited due to spatial separation of cells

RISK ASSESSMENT FOR ANTIBIOTIC RESISTANT PATHOGENS: Questions & Issues

- Do pathogenic ARB behave like non-ARB pathogens?

  We don’t know

- Is the dose response the same for ARB and non ARB pathogens?

  We don’t know, but some data suggest ARB pathogens more infectious than non-ARB

- Do ARB and non ARB pathogens survive similarly in the environment?

  Some ARB co-evolve mobile genetic traits (transposons and plasmids) that enhance metal resistance and other environmental tolerance. BUT plasmids are a genetic burden causing shorter survival duration.

RISK ASSESSMENT FOR ANTIBIOTIC RESISTANT PATHOGENS: Questions & Issues

- Does horizontal gene transfer (HGT) between ARBs and pathogenic bacteria in soil present a danger?

  HGT rates not known, but thought to be low

- Do ARBs maintain plasmid-borne resistance in soils?

  Unlikely without selective pressure

RISK ASSESSMENT FOR ANTIBIOTIC RESISTANT PATHOGENS: Questions & Issues

- Do fresh inputs of antibiotics into soil lead to enhanced ARGs?

  Not necessarily since gene clusters for antibiotic production always present in soil but only expressed when there are interactions with other microbes

- Based on issues, environmental fate and transport of ARBs and non-ARBs assumed to be identical… BUT at manifestation of disease, risk of morbidity and mortality can be altered

QMRA – *Salmonella* spp.

Assumptions
- >1 million total (ARB and non-ARB) annual cases (Scallan, 2011)
- 378 total annual deaths (Scallan, 2011)

Morbidity & Mortality

U.S. Population
- 2 per 1000 risk of infection/yr for crop ingestion (Brooks, 2012), thus
- ~200,000 infections/yr attributed to crop ingestion

~314 million

Disease Outcomes
- Mortality ~66 deaths/yr
- 0.036% of infections

QMRA – ARB *Salmonella* spp.

Assumptions
- 100,000 ARB annual cases (CDC, 2013)
- 40 ARB annual deaths (CDC, 2013)

Morbidity & Mortality

U.S. Population
- Assume risk of infection equivalent to non-ARB *Salmonella*
- ~1/10th of total cases are ARB (CDC, 2013), thus
- ~19,500 ARB *Salm* infections/yr

~314 million

Disease Outcomes
- Mortality ~8 deaths/yr
- 0.040% of infections

- Incidence of infections similar
- Disease outcome may be different if antibiotics are required
- Mortality rates similar because most *Salmonella* infections are self-limiting and don't require antibiotics
- For MRSA infections risk of mortality is greater

SUMMARY

- Based on:
  - Incidence of ARBs naturally present in soil
  - Numbers of added ARBs added via land application
  - Soil microbial ecology principles
- Current evidence suggests municipal waste applications to land do not cause any long term increase in ARBs or ARGs
- All soils currently contain large #s of indigenous antibiotic resistant pathogens – not a problem
- For formal risk assessment more research needed on fate and transport of ARBs

BIOSOLIDS ON TRIAL: REFLECTIONS ON THE KERN COUNTY CASE

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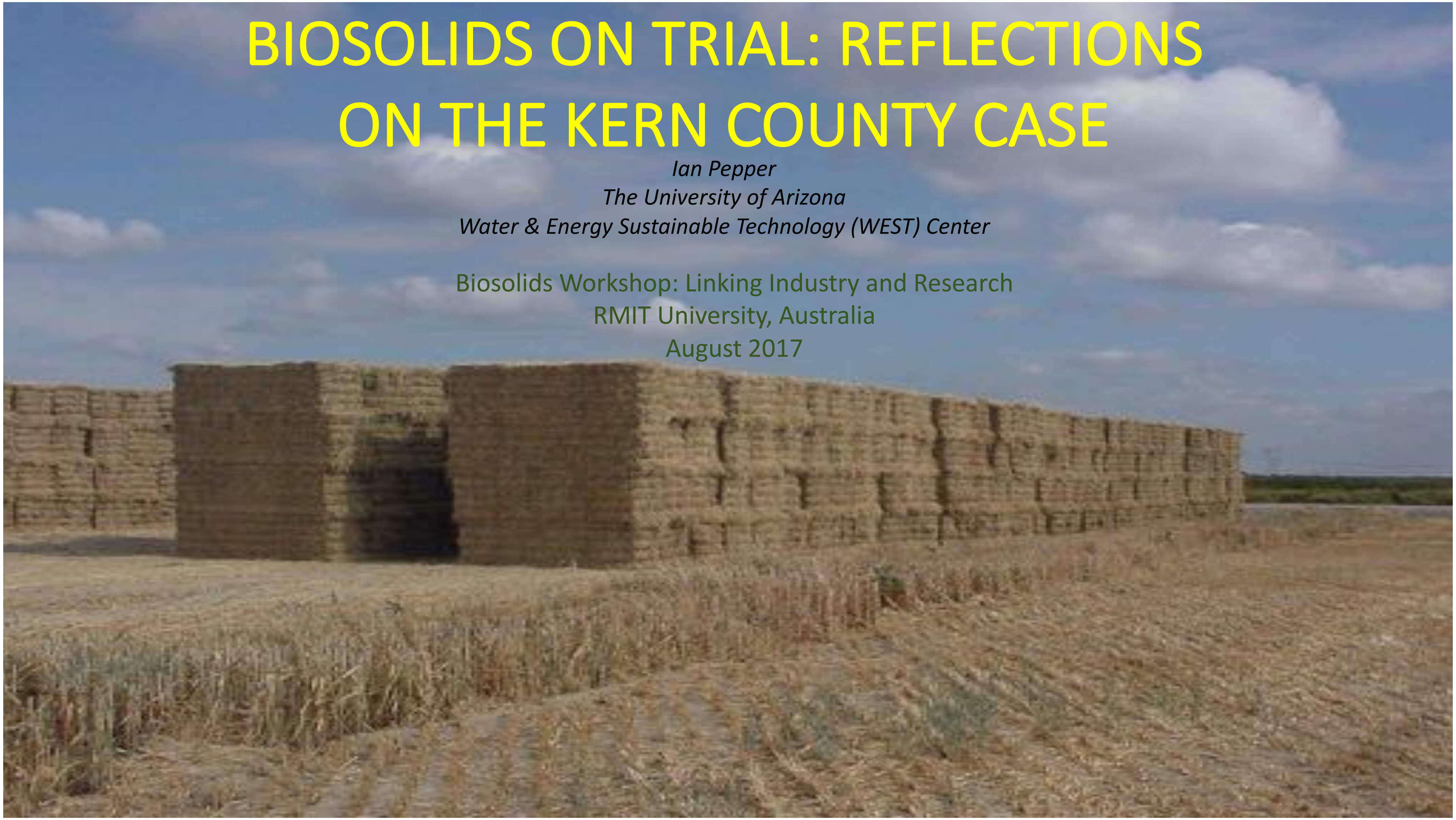
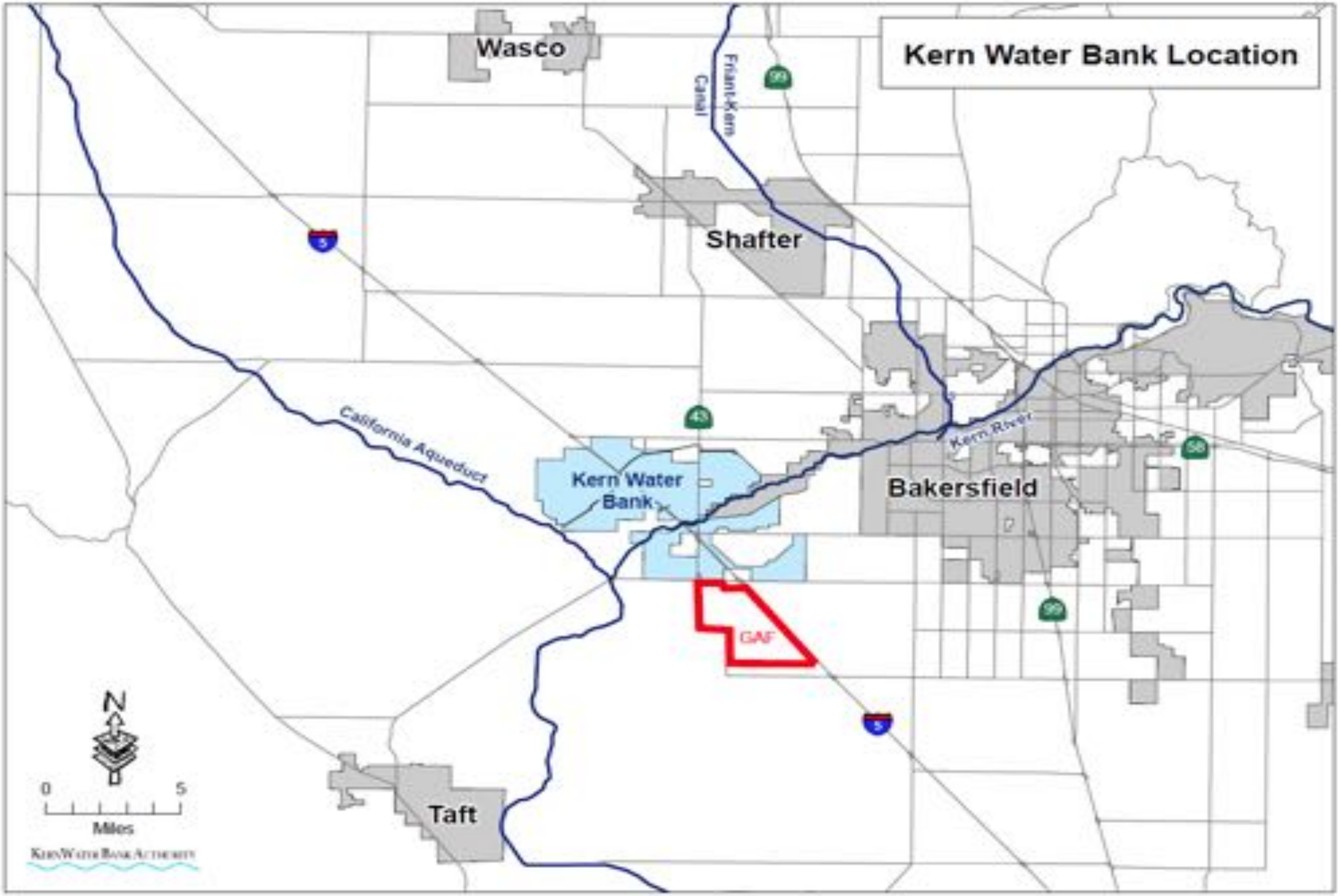



Kern Water Bank Location
Wasco
Shafter
Friant-Kern Canal
California Aqueduct
Kern Water Bank
Kern River
Bakersfield
GAF
Taft
N
0
5
Miles

Kern County Legal History

- Kern County passed ordinance in 1999 banning Class B land application beginning in 2003
- Class A only requirement adopted in 2003 with new ordinance
- Voters approved Measure E in 2006 which prohibited all land application in unincorporated parts of the County (did not impact Class B application in incorporated parts of county; e.g. City of Bakersfield

LA v. Kern: The Road to Trial

- Measure E voter initiative banning all biosolids in Kern County passed 2006
- Coalition of agencies, farmers and biosolids managers sued Kern County to overturn Measure E
- Measure E never went into effect; preliminary and permanent injunctions while case motions decided and appealed

LA vs Kern: The Road to Trial

- Measure E Challenged in State Court January 2011 after release of federal case

- Preliminary Injunction (PI) granted in June 2011

- PI upheld in Court of Appeals 2013

Biosolids Ban On Trial 2016

- Two week trial April-May 2016 in Tulare County Superior Court
- Four claims against Measure E:
  - Preemption of biosolids ban by IWMA
  - Ban exceeds limits on county police powers
  - Ban interferes with federal commerce
  - Ban interferes with state commerce

LA vs Kern Trial

- Eight days of evidence on biosolids safety from eight experts and twelve fact witnesses
- Kern County defense focused on trace perfluorinated compounds (PFCs) in groundwater
- Plaintiffs focused on no impacts after 22 years of land application

Experts for Kern

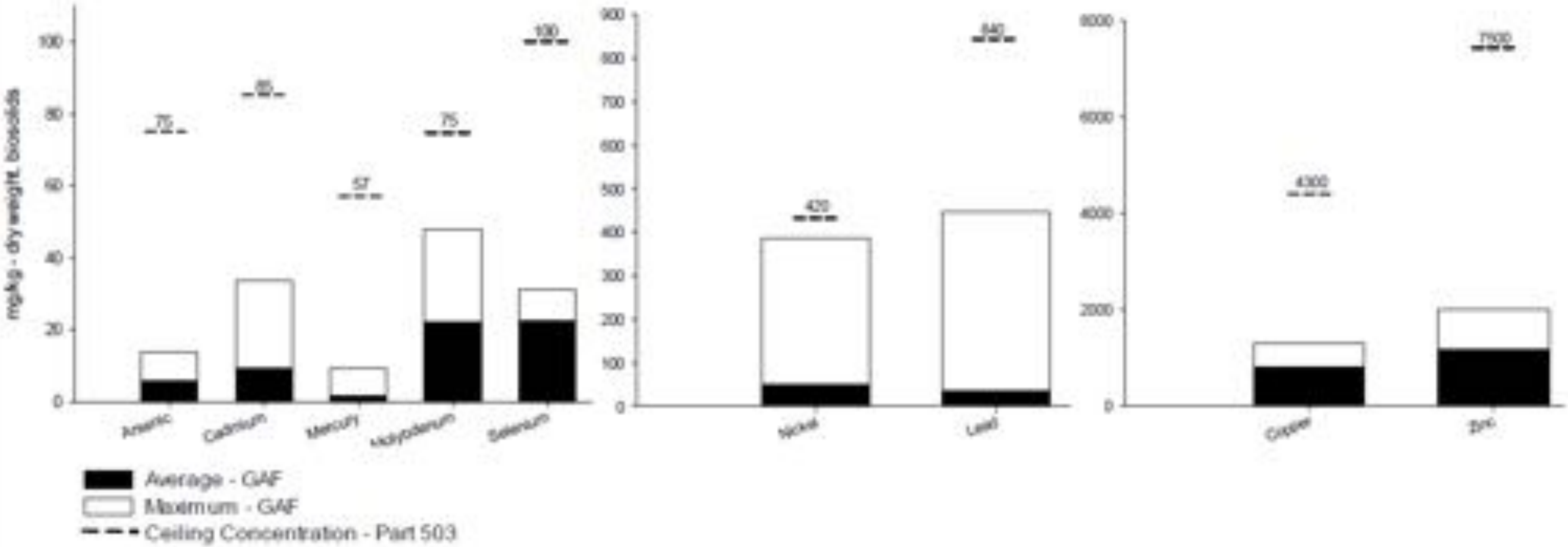
- Kern focused on trace organic compounds in soil and groundwater (PFCs) at ppb level, potential risks
- Chris Higgins (Colorado School of Mines)
- Gwynn Johnson (Portland State)
- Gary Hokkanen (hydrogeologist)

Key Witnesses for Plaintiffs

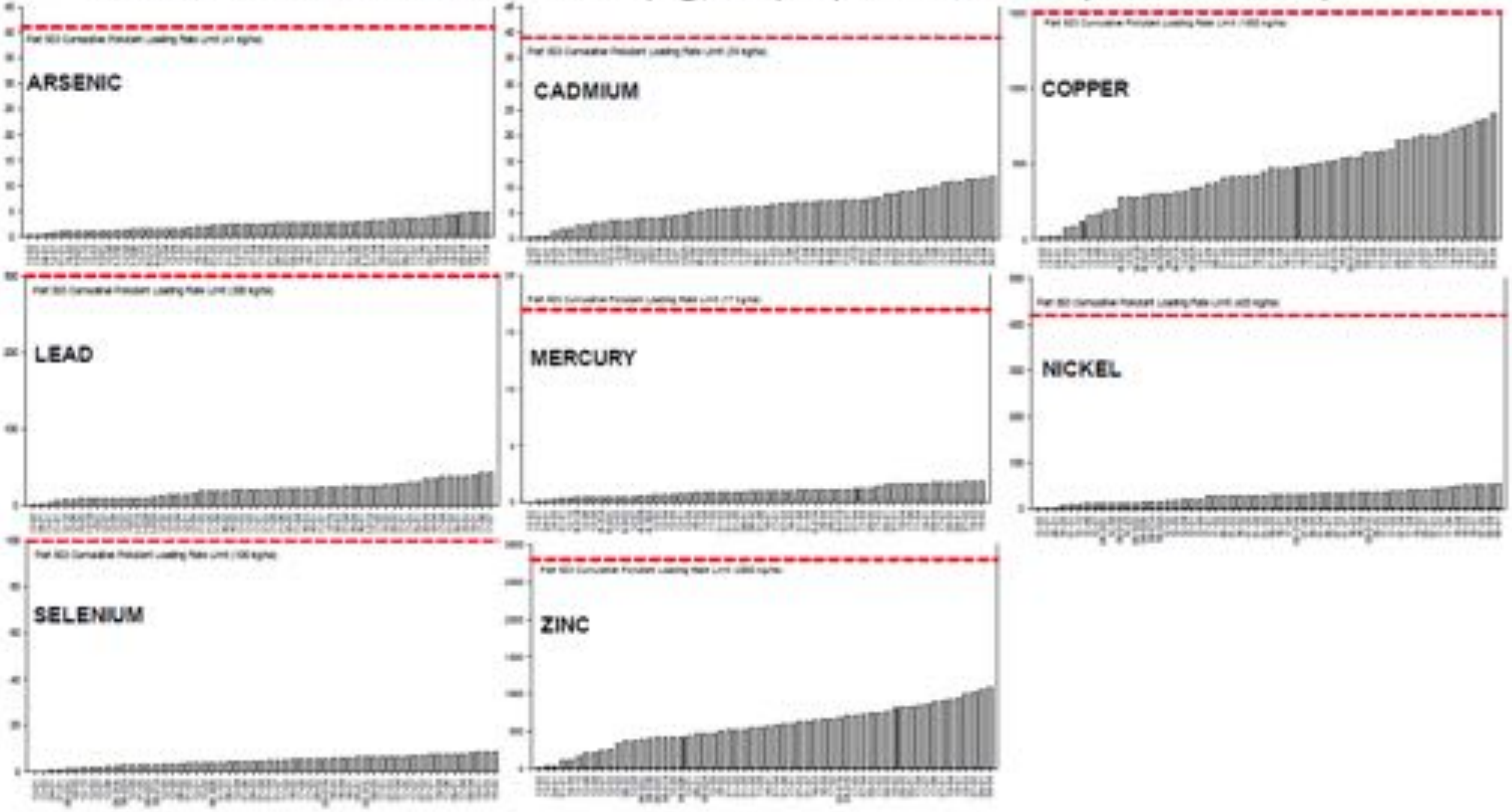
LA vs Kern Trial

- Trial extended over two weeks in April/May 2016
- Preliminary decision issued late November 2016
- Final decision in August 2017
- Ruled Measure E is invalid due to pre-emption of IWMA and exceedance of police powers
- Very strong and supportive decision
- Supports responsible management of both Class A and B

Metals in biosolids (1996-2015) applied at GAF did not exceed EPA Part 503 ceiling concentrations



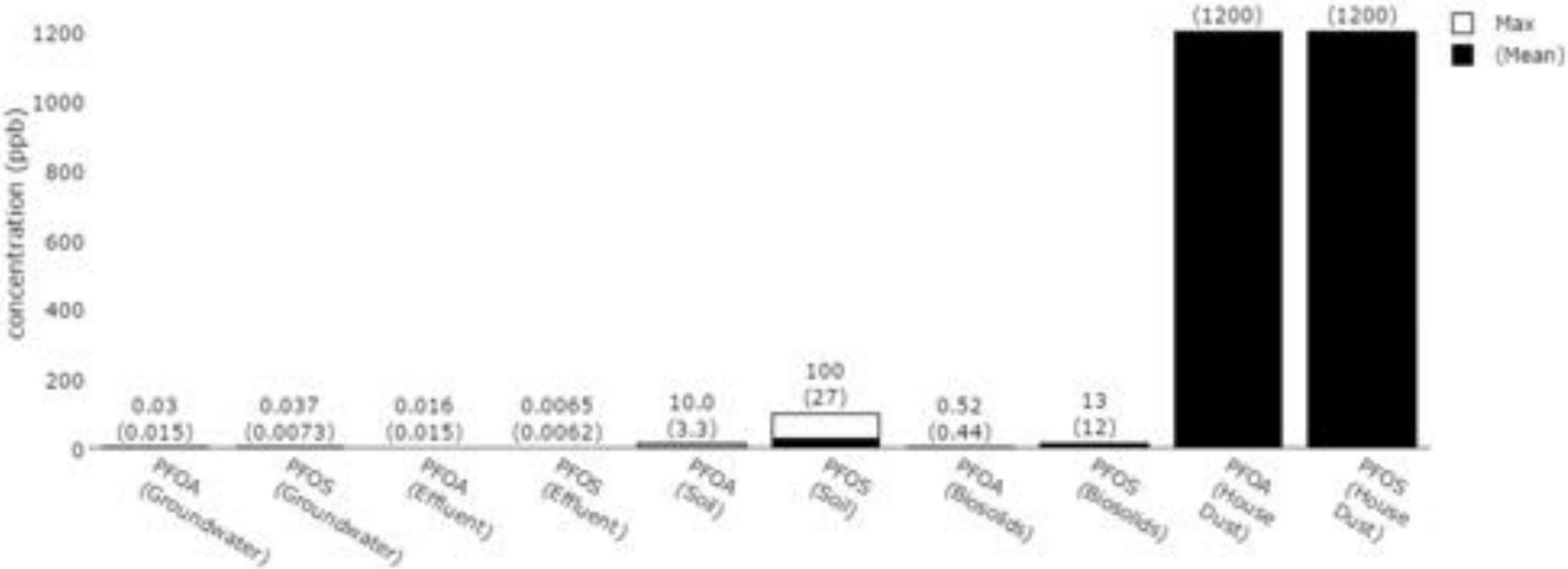
Current cumulative loads (kg/ha) by field, GAF (1996-2015)



PERFLUORINATED COMPOUNDS (PFCs)

- Fully fluorinated long chain organic compounds
- Family of anthropogenic chemicals used for decades to make products resistant to heat, oil stains, grease and water
- Perfluoroctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) produced in the largest amounts in the U.S.
- Regarded by EPA as an "emerging contaminant"

PFOA and PFOS in effluent, soil, and biosolids measured at Green Acres Farm, 2015, compared with PFOA and PFOS concentrations in household dust*

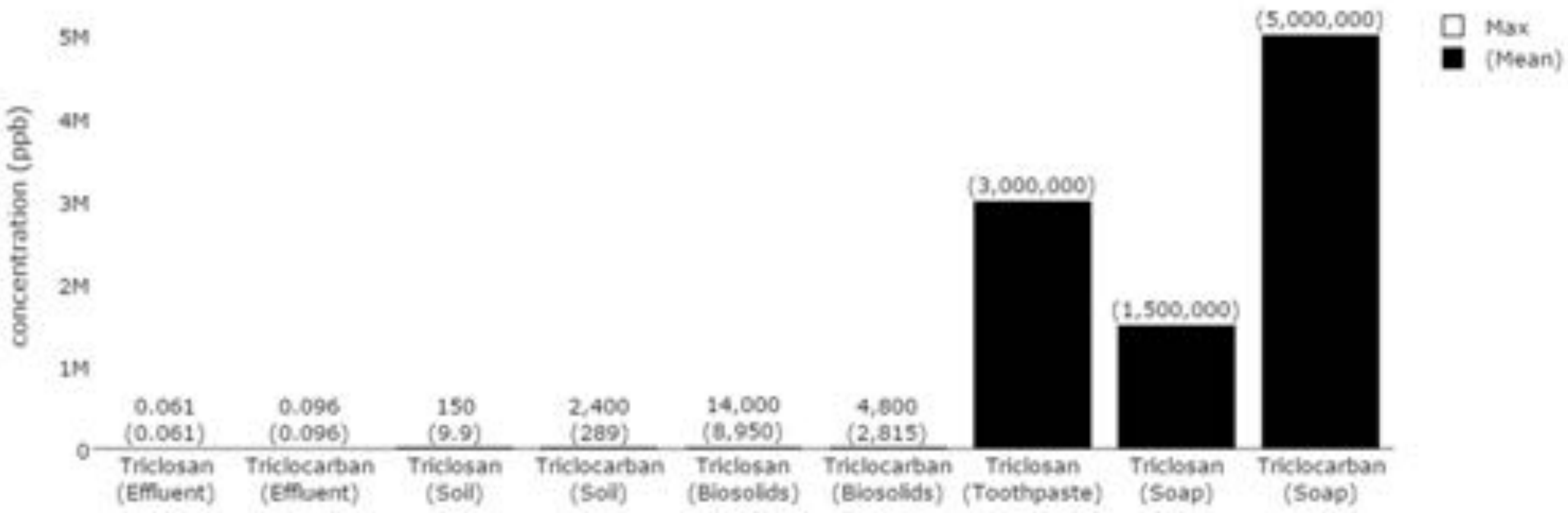


*Household dust measurements from Trudel et al. *Risk Analysis, Vol. 28, No. 2, 2008*

Triclosan and Triclocarban

- Common ingredients in every day cleaning products
    - Hand soaps
    - Dish and laundry soaps
    - Toothpaste
    - Mouthwash
- Triclosan shown to bioaccumulate in fish and humans
- Triclosan shown to be an endocrine disruptor in aquatic organisms
- Found in biosolids at the low ppm concentration
- Found in Colgate toothpaste at a concentration of 3000 ppm

Antimicrobials in effluent, soil, and biosolids measured at Green Acres Farm, 2015, compared with typical household products



Triclosan: toothpaste – Colgate 2016 (http://www.colgatetotal.com/health-benefits/colgate-total-triclosan);
Triclosan: soap – FDA, 2015 (http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm205999.htm)
Triclocarban: soap – OEHHA 2010 (http://oehha.ca.gov/multimedia/biomon/pdf/052410Triclocarban.pdf)

Judge Strikes Down Measure E November 2016 (preliminary decision)

- Measure E conflicts with Integrated Waste Management Act's mandate for recycling
- Measure E exceeds Kern County's police power because of impacts on neighbors, lack of benefits
- Court of Appeal reached same conclusions in 2013 when it upheld preliminary injunction

Opinion Stresses Safety of Land Application

- “There is no basis in fact for any determination that land application of biosolids poses any risk to Kern County”
- “Los Angeles has met its burden of producing evidence that there is no basis in fact for Measure E’s public welfare claims”
- “THERE IS NO EVIDENCE OF RISK TO HUMAN HEALTH”

No Reasonable Basis for a Ban

- “No real and substantial relationship” between a ban and the public welfare
- Measure E does not accommodate regional interests in cost-efficient management of biosolids
- No countervailing interests in Kern served by the ban

Next Steps for Protecting Land Application

- First trial on the safety of land application resulted in a resounding finding for benefits and lack of risk
- Kern County spent millions of dollars on sampling, experts, and one of country's largest law firms
- Trial court and appellate opinions should convince localities that future bans or regulations close to bans will be struck down at great expense to enacting jurisdiction

LA vs Kern

- Settlement reached August 2017 (Final Decision)
- Land application at Green Acres safe and will continue
- Land application elsewhere in Kern County still in dispute