Fm: Dr Edo McGowan

commentletters@waterboards.ca.gov

Re: Comments related to:

NOTICE OF PROPOSED RULEMAKING
TITLE 22. SOCIAL SECURITY
DIVISION 4. ENVIRONMENTAL HEALTH
CHAPTER 3
(Water Recycling Criteria)
&
CHAPTER 17
(Surface Water Treatment)

SUMMARY This brief is designed as a primer for planners and policy makers not familiar with the interaction of sewage byproducts and disease. The paper presents an argument that accelerating risks from antimicrobial resistance, virulence, and pandemic, especially as now found emerging in the world community, may be related to the disposal of inadequately treated sewage and its byproducts, including recycled water. Sewage may be utilized in different forms, such as solids (sewage sludge, now termed by EPA as biosolids), or liquids for irrigation via recycled water used on vegetable crops or municipal greenscape. On most continents, the practice of land applied sewer sludge or irrigation with recycled wastewater has gained unprecedented acceptance based on the need to rapidly rid ourselves of our waste or augment potable water supplies. In the drought-prone areas, there is much interest in recycled sewage effluent for offsetting use of potable water. These varied uses of sewage and its byproducts are not without increased public health risk.

Recent papers have noted the mixing of genetic material between various organisms provides for new types of pathogens or rears existing pathogens with new capacities for disease. Consequently, through these changes our immune systems may be faced with an unknown foe for which it is ill prepared, if prepared at all. Interestingly, much of this genetic mixing goes on every day in sewage treatment plants in almost every city. Further, the impact of disinfecting with chlorine enhances virulence as well as creating resistant bacteria. These virulence enhanced and resistant bacteria adversely impact the immune system and may render existing antimicrobial drugs useless. In fact giving antimicrobial drugs in such cases may actually exacerbate the disease process. Millions of gallons of effluent and tons of poorly treated sewage wastes are discharged to the environment and the solids, now termed biosolids are spread across thousands of square miles of farmland. There, these materials are open to background organisms, thus allowing the intermixing with numerous species at the micro and macro biological levels. Regulatory agencies are apparently ignorant of these issues, notwithstanding a long history of competent literature discussing such issues.

Until policy-makers become aware of these failings by the regulatory agencies, the world will see an
advancing acceleration of antibiotic resistance with concomitant amplified health care costs.

It is generally believed that the overuse of antibiotics has driven antibiotic resistance. Serious antibiotic resistant pathogens are no longer restricted to the very ill within the confines of hospitals; these pathogens are now commonly found within the community. Some have gained sufficient resistance and virulence to make them invincible to all currently available antibiotics and medical interventions; the result is seen as increasing and unnecessary morbidity and mortality. Some now need antibiotics to even exist. Others are made more virulent through treatment with disinfectants.

While over-use of antibiotics may play an important role in the advancement of resistance, other causes of resistance to antibiotics are overlooked. A critical but less well understood mechanism that initiates the transfer of genetic information conferring resistance and virulence, thus amplification of pathogenesis, is found at the local sewer-treatment plant [1]. As bacteria, other pathogens, and common background-organisms wind their way through municipal sewage treatment processes there is the intermixing of vast quantities of organisms that might otherwise never come together. In the process of sewage treatment, the selective pressures against these organisms is increased. In consequence, there is a greater effort by these organisms to up-regulate or acquire numerous other survival mechanisms to assure that they and their genetic material persist to pass on genetic information. Part of this defense strategy is to go dormant—shut down metabolic processes, and enter a viable but non-culturable (VBNC) state. In this VBNC state these organisms are essentially invisible to standard laboratory tests. Other consequences include the toxicogenomic response to challenges such as disinfection, chlorine being a major effector in such genenomic shifts that enhance virulence (MW Chang 2007).

Additionally, as the environmental crowding and stresses increase, these organisms can acquire and then pass on to other non-related organisms the acquired antibiotic resistance, virulence, as well as resistance to heavy metals and chlorine. In many cases, these changes to the cellular and metabolic machinery afford the ability to deal with numerous insults, hence development of cross-resistance mechanisms. How does a sewer plant acquire this capacity? Many antimicrobials or their metabolites pass through the body essentially unchanged. Thus feces and urine do contain some impressive levels. As later noted, Kümmerer and others (1999, 2000, 2003, 2004) [2], have followed this and have noted levels of antibiotics in sewage that are able to induce or maintain resistance. Added to this are the other materials dumped into the toilet or down the drain that confer resistance. This includes discarded antibiotics and disinfectants such as Triclosan [3] a ubiquitous biocide has been suspected of inducing resistance.

Based on wastewater industry dogma and standards, released effluent, its use in irrigation salad crops, and the land application of sewage sludge are benign and beneficial activities. If however, one reviews the current medical and scientific literature, a different picture emerges, one that raises serious questions about the benevolence of this activity and efficacy of the underlying standards. Thus, the issue takes on aspects of a political and not a scientific argument. In the interim, most regulatory agencies have backed off [ ]. Recent court documents also tend to implicate that high-level officials within EPA may have conspired with sludge industry scientists to falsify data and thus to deliberately obscure the adverse health impacts from land applied sludge (see: Qui Tam Complaint via http://www.sludgefacts.org/). This leaves the citizens and patient base standing naked.

In one of several major studies looking at sewage treatment plants, the scientists followed bacteria through a sewer treatment works using fecal coliforms as the test organism [4]. Coliform bacteria were isolated at various locations in the plant, specifically a) the inlet, b) the primary sedimentation tank, c) the activated sludge digestion tank, d) the final settling tank, e) the outlet and f) the return activated sludge drain. They were then examined the presence of drug resistant plasmids. Using this approach, resistant bacteria and
those that were still sensitive to antibiotics were detected [5].

Several drugs were tested and included tetracycline, kanamycin, chloramphenicol and streptomycin, ampicillin, nalidixic acid, rifampicin, and sulfisoxazole. A total of 900 separate tests were conducted, of which more than half contained multi-drug resistant plasmids. While this is interesting, there was a new finding that raised considerable concern. The further along that the wastewater had progressed through the treatment process, the greater the tendency was to encounter strains that had developed multiresistance to antibiotics. Additionally, the study demonstrated that these multi-resistant bacteria also simultaneously carried, and then passed around their multiple transferable drug-resistance plasmids. Thus, the development of drug resistance and the transfer of multi-drug resistance are enhanced in sewage wastewater treatment plants [5]. These findings have been documented for more than a decade. They were a harbinger, yet little impact from such studies has been noted. Under the current practices, sewage treatment practices allow the survival of up to 2-million viable coliform per gram of sludge at the point of land application to farmlands. The new WERF paper by Higgins and Murthy (2006) now raises some serious questions about the efficacy of current standards. These authors noted that in the centrifuge dewatering of sewer sludge the indicators were in a VBNC state and thus centrifuging resuscitated them. The numbers were several magnitudes greater that standard plate count would have indicated. [6]. This finding by Higgins and Murthy should raise some logical questions. For example, if the dewatering by centrifuge brought out the essence of VBNC, then were those sludge lots not subjected to the centrifuge also in the VBNC state and thus would revive in the field. That may explain why we see large and rapid regrowth of pathogens following land application. Further logical questions might be—what of the more robust pathogens—pathogens not easily killed by the low-level disinfection attainable within sewer currently operated plants.

Additionally, we are seeing resistance developing to chlorine in tests made on bacteria coming from sewage plants. Chang, et al, (2007), notes that exposure to chlorine of Staphylococcus aureus causes shifts in the genes, thus bringing out several virulence factors. As noted above, numerous pathogens survive their passage through sewage treatment. Further, it is well known that the human immune system relies on the capacity of the white blood cells to kill bacteria by engulfing them and then subjecting these bacteria to a bath of hypochlorite—a chlorine derivative. Thus, through exposure of pathogens to sewage treatment, the resultant augmentation of virulence and chlorine resistance poses a direct threat to the human immune system. This threat is compounded by the fact that numerous antibiotics are merely bacteriostatic, i.e., these antibiotics don’t kill bacteria, they merely arrest them while awaiting the immune system to work. Thus a compromised immune system along with antibiotics may actually exacerbate the disease process, as noted by Chang.

The use of low-level indicator bacteria, along with the apparent lack in understanding of antibiotic resistance within EPA (see FOIA search results at bottom of this file) should alert anyone that the issue is anything but closed. By its refusal to adequately present necessary analyses in this area of antibiotic resistance, EPA has not only manufactured uncertainty, but also potentially increased the risk of human disease, disease from some serious pathogens that may not respond to current antibiotics.

Sewage sludge, or as industry calls it, biosolids, is the residual solids that are separated from the raw sewage during sewage treatment. There are two basic classes of sewage sludge, Class A, which is presumed to be essentially free of pathogens, but in reality may contain significant numbers. The other is Class B, which is acknowledged to contain pathogens. The majority of land applied sewage sludge is Class B.

All Class-B sewage sludge technologies that are normally used in the U.S. such as anaerobic digestion and aerobic digestion and heating at these levels as well as composting and land stabilization do not effectively destroy critical pathogens [7]. These practices also do not destroy the genetic material and this and its lack of acknowledgement is a critical shortcoming within EPA. Thus if there is antibiotic resistance within sewer sludge, it may be passed through these processes to background organisms including man [8]. Actually
several studies have documented the horizontal transfer of genetic information to background environmental systems and such systems can act as lending libraries for this genetic information. Man and animals are exposed daily to such backgrounds [9].

The same can be said for reclaimed (recycled) wastewater. Thus far, my group has tested recycled water from 6 sewer plants in California. All showed resistant bacteria, some resistant to vancomycin, the most popular drug used for methicillin resistant Staphylococcus aureus (MRSA). The resistance to vancomycin is not unexpected because the cell walls of gram negative are able to exclude the rather large molecular structure. All sewer plants in California work off the same state criteria for the production of recycled water. These criteria do not consider antibiotic resistance, transfer of genetic information nor do the lab tests consider viable but non-culturables. Thus, it is not unreasonable that my group is finding resistant organisms in the recycled water. Joan B Rose, looking at recycled water via a Water Environment Research Foundation study (2004) noted that sewer plants tested in Florida, Arizona, and California all contained pathogens. Giardia cysts were found in 84% of the final treated water. Enteric viruses were found in 31% of the final product in 2/3 of these plants and Cryptosporidium were noted in 71% of the final product of all tested plants. Thus pathogens and resistant pathogens do get through into the environment where niches can be established and these may act as lending libraries. It is important to remember that reclaimed water receives treatment that is far more rigorous than sewage sludge. Thus if we are finding these levels of pathogens in reclaimed water, what would we find in sewage sludge?

Amy Pruden, et al. (2006) followed genetic information through sewer plants into the open environment and thence into the drinking water supply. The genetic information is not inhibited by chlorine since it is not alive, in the sense of a living cell. Also because of its small size, it slips through most of the current filtering systems utilized by recycled water or drinking water treatment systems.

As to viruses, for example, the French government, took action to reduce the risk of viral contamination associated with land application of sewage sludge. It passed legislation (decree of 8 January 1998 related to the landing of sewage sludge on agricultural soils) requiring that microbiological testing be carried out for validation of stabilization processes. This differs from the U.S. system which is essentially a voluntary self policing by the industry and has notoriously poor follow up by the regulatory bodies. The virological testing method currently specified in the French system is based on the counting of enterovirus particles. Unlike the U.S, the French government passed a national health care coverage system where victims of sludge illness are covered.

What are the chances for inadvertent acquisition of resistance from environmental contamination such as through sewage sludge or recycled water? Gerba and Rusin [10] conducted research about the passage from finger to mouth of pathogens found on typical household objects. Others have documented dust as a mechanical vector for pathogens. Thus what of the dwellings and towns down wind from land application of sewer sludge, from a sewage sludge composting facility, or sprinkler irrigation with recycled water? In the last case, drift at night when many municipal programs use recycled water to irrigate neighborhood parks, finds drift and survival of pathogens enhanced. Considering the proximity of residences adjacent to public parks, just across the street, the distances may not be sufficient to assure public health protection. In the arid portions of the country, and during the summer when night irrigation is underway, many windows are open. In a German study, the recommended setback was 300 meters between sprinklers and human settlements. This is hardly the case in most American cities. Please remember that the German government, unlike the U.S. system, is the responsible party for health care.

With respect to dust from sewage sludge composting, there are now several workmen’s comp cases filed by staff of the Chino Women’s Prison for complaints accruing to dust from the adjacent and up-wind sewer sludge composting facility in San Bernardino County, California. This access to medical coverage is accorded to employees but not inmates.

Further, there are concerns about contamination through wash-off from rains and irrigation return flows.
Gerba and others have written extensively about the survival of pathogens and their viable infectivity once they are adsorbed onto sediments [11]. While this work by Gerba related to marine sediments, similar conditions need to be evaluated for fresh water systems. Selvaratnam and Kunberger (2004) looked at the off-site movement of antibiotic resistance into adjacent water bodies from sewage sludge applied fields. These authors suggest that surface runoff from the farmland is strongly correlated with higher incidence of resistant genes and pathogens found in the adjoining water bodies, in this case recreational water that is tributary to drinking water sources.

Anyone who lives in an agricultural area knows that tillage and wind cause large movements of soil and dust that are equal to that found for water erosion. The USGS has written extensively on the movement of dust from Africa, across the Atlantic and carrying with it viable pathogens thus causing respiratory disease in the Caribbean [12].

The indicator organisms used for Class B biosolids commonly include Escherichia coli and sometimes Salmonella. These are the organisms that are normally killed by low-level disinfection. They are vegetative bacteria that are highly susceptible to both chemical disinfection and heat disinfection. However, sewage sludge contains a large range of organisms besides E. coli, Salmonella, and Staphylococcus. Also highly susceptible and easily inactivated are the enveloped viruses such as Hepatitis B., HIV, and influenza. While these organisms are fairly easily destroyed, Class-B allows 2 million viable coliform per gram of land applied sewer sludge. This raises the logical question of survival for the more robust organisms. The non-enveloped viruses are hard to kill. Pathogens that require high-level disinfection are missed by sewage treatment processes. These are those pathogens that contaminate semicritical medical devices such as the scopes inserted into the lower bowel. No sewer treatment plant reaches high-level disinfection.

These bacteria when released by sewage treatment or contained within sewage byproducts are thus able to colonize environmental niches, and animals, including humans, through ingestion. Once ingested, the plasmids may be transferred to normal flora, and subsequently to pathogenic bacteria found in humans or animals, making later treatment with particular antibiotics ineffective. Also one must consider transfer of genetic information from these organisms to more robust organisms as highlighted by Sjolund et al. (2005) [13] indicating that resistance in the normal flora, which may last up to four-years, might contribute to increased resistance in higher-grade pathogens through interspecies transfer.

Sjolund et al go on to note that since populations of the normal biota are large, this affords the chance for multiple and different resistant variants to develop. This thus enhances the risk for spread to populations of pathogens. Furthermore, there is crossed resistance. For example, vancomycin resistance may be maintained by using macrolides [14].

Walsh (2003) [15] notes that resistance to antibiotics is not a matter of IF but one of WHEN. Schentag, et al. (2003), as found in Walsh, followed surgical patients with the subsequent results. Pre-op nasal cultures found Staphylococcus aureus 100% antibiotic susceptible. Pre-op prophylactic antibiotics were administered. Following surgery, cephalosporin was administered. Ninety percent of the patients went home at post-op day 2 without infectious complications. Nasal bacteria counts on these patients had dropped from 10/5th to 10/3rd, but were now a mix of sensitive, borderline, and resistant Staphylococcus sp. By comparison, prior to surgery, all of the patients’ Staphylococcus samples had been susceptible to antibiotics. For the patients remaining in the hospital and who were switched on post-op day 5 to a second generation cephalosporin (ceftazidine), showed bacterial counts up 1000-fold when assayed on post-op day 7 and most of these were methicillin resistant Staphylococcus aureus (MRSA). These patients were switched to a 2-week course of vancomycin. Cultures from those remaining in the hospital on day 21, revealed vancomycin resistant enterococcus (VRE) and candida. Vancomycin resistant enterococci infections can
produce mortality rates of between 42 and 81%.
Note in the above, that these patients harbored NO resistant bacteria in their nasal cavities upon entry to
the hospital. But what would be the result if there had been inadvertent acquisition of resistance from
environmental contamination such as through sewage sludge or recycled water

This then brings into question the current paradigm on infection and its dose response to a certain load of a
particular pathogen, i.e., ID and LD 50s. Lateral transfer of mobile genetic elements conferring resistance is
not considered in this old paradigm. With the prodigious capacity for the gut bacteria to multiply, once the
lateral transfer has taken place, very small original numbers—well below the old paradigms can be
multiplied into impressive numbers. Since viruses and phages are also involved, their capacity to multiply,
which dwarfs that of bacteria, must also be included. Thus there is a need for a new paradigm;
unfortunately, the regulatory community seems not to recognize this. When one considers the
multiplication within sewer plants and also within their byproducts, disbursement into the environment, the
transfer to background organisms, hence to man and his animals, then the remultiplication within
commensals, the emerging picture is worrisome.

Further, there are opportunities and interrelationships between microbes that can degrade antibiotics, eg.
antibiotic resistant bacteria, and those that can degrade metals as well as pesticides and farm chemicals that
are already found in agricultural soils. In many cases, the involved cellular machinery is the same or similar,
i.e., a duality (see Schlüter and abstracts of others below).

This duality may have some interesting synergistic survival advantages for the microbes, but bad-for-human-
health effects when considering sewer sludge as applied to heavily farmed lands.

The current standards controlling sewer plant operations, the land application of sewer sludge or the
composting of sewer sludge for making compost and potting soils consider none of these issues. This paper
therefore contends that this unconsidered avenue for the spread of antibiotic resistance and amplification
of risk for a pandemic needs greater awareness within the medical and health care community.

Perhaps this is an area worthy of further review by policy committees. Without the perspective of a broader
analysis of this issue, future policy may be no more that the post hoc rationalization for a series of missed
opportunities. It would seem reckless to proceed without a broader picture. Unfortunately, the principal
regulatory body, U.S. EPA seems to be essentially oblivious to these concepts, yet it has been promoting the
land application of sewer sludge. As seen below, based on a FOIA request, EPA seems less than
knowledgeable in the area of antimicrobial resistance.

Citations and notes
Fontaine TD et al. Transferable drug resistance associated with coliforms from hospital and domestic
oxtetracycline resistance plasmids between aeromonads in hospital and aquaculture environments:
implication of Tn1721 in dissemination of the tetracycline resistance determinant tet A. Appl Environ


[13] Sjolund et al. (2005) Emerging Infectious Diseases (Vol. 11, # 9, Sept 2005 @ p. 1389 et seq),


+++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

In February 2005, my group had requested, via Freedom of Information Act, certain data from the U.S. EPA on their progress dealing with biosolids and resistance. In providing us answers to this request, EPA delayed its response for about 6 months and then merely directed us to a section of the NERL’s website, which contained no usable information. This site was (www.epa.gov/nerlesd1/chemistry/pharma/fq.htm#disposal), as evidenced by the following search results. Similar results were found for other EPA web addresses. It is now nearly two years and EPA has yet to respond.

Results of Searching the “Environmental Sciences” Area of EPA’s Web Site
No matches found for transposon; 1402 files searched
No matches found for antibiotic resistance + biosolids; 1402 files searched.
No matches found for antimicrobial resistance + biosolids; 1402 files searched.
No matches found for virulent pathogens + biosolids; 1402 files searched.
No matches found for plasmids + biosolids; 1402 files searched.
No matches found for mobile genetic elements; 1402 files searched.
No matches found for high level disinfection + biosolids; 1402 files searched.

Results of Searching EPA's Entire Web Site
We have searched the entire EPA site and found the following results. You may also return to searching for the same terms within Environmental Sciences.
No matches found for high level disinfection + biosolids; 494732 files searched.
No matches found for plasmids + biosolids; 494732 files searched.
No matches found for transposons + biosolids; 494732 files searched.
No matches found for mobile genetic elements + biosolids; 494732 files searched.
No matches found for virulent pathogens + biosolids; 494732 files searched.
No matches found for antibiotic resistance + biosolids; 494732 files searched.
The 64,508 bp IncP-1 antibiotic multiresistance plasmid pB10, isolated from a waste-water treatment plant provides evidence for recombination between members of different branches of the IncP-1 group. A. Schlüter, et al.

The complete 64,508 bp nucleotide sequence of the IncP-1 antibiotic-resistance plasmid pB10, which was isolated from a waste-water treatment plant in Germany and mediates resistance against the antimicrobial agents amoxicillin, streptomycin, sulfonamides and tetracycline and against mercury ions, was determined and analysed. A typical class 1 integron with completely conserved 5’ and 3’ segments is inserted between the tra and trb regions. The two mobile gene cassettes of this integron encode a -lactamase of the oxacillin-hydrolysing type (Oxa-2) and a gene product of unknown function (OrfE-like), respectively. The pB10-specific gene load present between the replication module (trfA1) and the origin of vegetative replication (oriV) is composed of four class II (Tn3 family) transposable elements: (i) a Tn501-like mercury-resistance (mer) transposon downstream of the trfA1 gene, (ii) a truncated derivative of the widespread streptomycin-resistance transposon Tn5393c, (iii) the insertion sequence element IS1071 and (iv) a Tn1721-like transposon that contains the tetracycline-resistance genes tetA and tetR. A very similar Tn501-like mer transposon is present in the same target site of the IncP-1 degradative plasmid pJP4 and the IncP-1 resistance plasmid R906, suggesting that pB10, R906 and pJP4 are derivatives of a common ancestor. Interestingly, large parts of the predicted pB10 restriction map, except for the tetracycline-resistance determinant, are identical to that of R906. It thus appears that plasmid pB10 acquired as many as five resistance genes via three transposons and one integron, which it may rapidly spread among bacterial populations given its high promiscuity...

Risk Assessment

A Dynamic Model to Assess Microbial Health Risks Associated with Beneficial Uses of Biosolids - Phase 1
John M. Colford*, Jr, Don M. Eisenberg**, Joseph N.S. Eisenberg*, James Scott* and Jeffrey A. Soller**

ABSTRACT
Maximum allowable levels for chemical contaminants in biosolids were developed for the Part 503 rule using risk-based methodologies. However, maximum allowable levels of microbiological contaminants in the Part 503 rule were based on specific treatment methodologies rather than risk levels, because it was determined at that time that risk assessment methodologies were not sufficiently developed. Given the current interest in the beneficial uses of biosolids and the projected rapid growth of biosolids reuse, there is increasing
interest in the development of a microbial risk assessment methodology for regulatory and operational decision making. This document presents a methodology for assessing risks to human health from pathogens via exposure to biosolids. The methodology integrates two fundamental components: an exposure assessment component and a health risk component. The exposure assessment component is used to quantify pathogen levels in the environment and serves as input to the health effects component. The health effects component is used to quantify health risks using a model that explicitly accounts for properties unique to an infectious disease process, specifically secondary transmission and immunity.

To demonstrate the applicability of these risk-based methods developed for biosolids exposure, numerical simulations were carried out for a case study example in which the route of exposure was direct consumption of biosolids-amended soil. [McGowan's comment interjected---what about respiratory or the interaction of contained chemicals on the inflammatory reaction, thus breaking barriers?] The output from the case study yielded a decision tree that differentiates between conditions in which the risk from biosolids exposure is high and those conditions in which the relative risk from biosolids is low. This decision tree illustrates the interaction among the important factors in quantifying risk. For the case study example, those factors include biosolids treatment processes, the pathogen shedding rate of infectious individuals, secondary transmission and immunity. Further work in determining biosolids exposures is required before this methodology can be used in a comprehensive risk assessment.

McGowan's comment---EPA which controls he land-spreading of sewage sludge has never done a health risk assessment on pathogens. The interesting thing here is that in relying on technology, that technology has failed to consider the viable but non-culturable (VBNC) aspect as well as persisters and biofilms. Further, as noted by the recent paper by Higgins & Murthy, pathogens in the VBNC state are missed by standard lab analysis, hence potentially vastly understating actual risk. Their paper noted that using centrifuges to dewater sewage sludge rather than belt presses saw bacterial counts rocket within 20 minutes to several magnitudes above what the lab had just noted.

Additionally, where is the potential for transfer of antibiotic resistance from a very small number to the gut bacteria and then its (the gut bacteria's) prodigious capacity to multiply that information? Thus their whole study---absent this aspect is badly flawed. Reliance on this study would then vastly underestimate the real risks. ---See: Maria Sjölund's paper below indicating a long-standing ability for these bacteria to remain in the gut .

"resistant strain may persist for 4 years, in the absence of further antimicrobial treatment."

Also, for example, Levy found that the resistance in gut bacteria of cattle moved to gut bacteria of mice having access to the same area, then from the mice to pigs, chickens,
infectious disease process, specifically secondary transmission and immunity. To demonstrate the applicability of this risk-based method, numerical simulations were carried out for a case study example in which the route of exposure was direct consumption of biosolids-amended soil and the pathogen present in the soil was enterovirus. The output from the case study yielded a decision tree that differentiates between conditions in which the relative risk from biosolids exposure is high and those conditions in which the relative risk from biosolids is low. This decision tree illustrates the interaction among the important factors in quantifying risk. For the case study example, these factors include biosolids treatment processes, the pathogen shedding rate of infectious individuals, secondary transmission, and immunity. Further refinement in methods for determining biosolids exposures under field conditions would certainly increase the utility of these approaches.

McGowan's comments on the Risk Analysis paper------
A brief read of this paper produced the following comments. Principal amongst my thoughts is the paper’s limit to pathogens that would not likely multiply outside the host---i.e., viruses. Thus, the model is quite limited from this important perspective. Secondly, there is no consideration of transfer of mobile genetic elements (MGEs) to terrestrial reservoirs, the potential for shifts in genetic information passing through multiple species, and thus the potential for newly emerging diseases. Consequently the issue of transferred antibiotic resistance and similar molecular and cellular machinery is missed. They also do not discuss colonization or later acquiring of resistance, the fecal veneer and thus movement into other organ systems or orifices.

+++++++++++++++++++++++++
Hospital effluent: A source of multiple drug-resistant bacteria
V. Chitnis, D. Chitnis*, †, S. Patil** and Ravi Kant*

The present work was carried out to study the spread of multiple drug-resistant (MDR) bacteria from hospital effluent to the municipal sewage system. The MDR bacteria population in hospital effluents ranged from 0.58 to 40% for ten hospitals studied while it was less than 0.00002 to 0.025% for 11 sewage samples from the residential areas. Further, the MDR bacteria carried simultaneous resistance for most of the commonly used antibiotics and obviously the spread of such MDR bacteria to the community is a matter of grave concern.

++++++++++++++++++++++++++++++=
Increased frequency of drug-resistant bacteria and fecal coliforms in an Indiana Creek adjacent to farmland amended with treated sludge

Shivi Selvaratnam and J. David Kunberger

Abstract: Many studies indicate the presence of human pathogens and drug-resistant bacteria in treated sewage sludge. Since one of the main methods of treated sewage disposal is by application to agricultural land, the presence of these organisms is of concern to human health. The goal of this study was to determine whether the frequency of drug-resistant and indicator bacteria in Sugar Creek, which is used for
recreational purposes, was influenced by proximity to a farmland routinely amended with treated sludge (site E). Surface water from 3 sites along Sugar Creek (site E, 1 upstream site (site C) and 1 downstream site (site K)) were tested for the presence of ampicillin-resistant (AmpR) bacteria, fecal and total coliforms over a period of 40 d. Site E consistently had higher frequencies of AmpR bacteria and fecal coliforms compared with the other 2 sites. All of the tested AmpR isolates were resistant to at least 1 other antibiotic. However, no isolate was resistant to more than 4 classes of antimicrobials. These results suggest that surface runoff from the farmland is strongly correlated with higher incidence of AmpR and fecal coliforms at site E.

Key words: drug-resistant bacteria, indicator bacteria, treated sludge, surface runoff.

Antibiotic resistance genes (ARGs) should be considered emerging environmental contaminants with more research devoted to the mechanisms by which they spread, scientists say in a report scheduled for the Dec. 1 issue of the semi-monthly ACS journal Environmental Science & Technology. Colorado State University's Amy Pruden and colleagues reached that conclusion after a study that documented occurrence of tetracycline and sulfonamide Antibiotic resistance genes in irrigation ditches, river sediments, and other spots in the environment in northern Colorado. They detected tetracycline resistance genes in treated drinking water, suggesting that it may be a pathway for spread of Antibiotic resistance genes to humans.

Antibiotic resistance genes are pieces of DNA that make bacteria resistant to common antibiotics - recognized as an increasingly serious global health problem. The genes can spread in different ways. Bacteria, for instance, exchange Antibiotic resistance genes among themselves. Pruden and colleagues note that even if cells carrying ARGs have been killed, DNA released to the environment can persist and spread to other cells. "ARGs in and of themselves can be considered to be emerging 'contaminants' for which mitigation strategies are needed to prevent their widespread dissemination," they state.

The Importance of Municipal Sewage Treatment in the Spread of Antibiotic Resistance
106th General Meeting of the American Society for Microbiology
May 21-25, 2006, Orlando, Florida
For more information on any presentation at the 106th General Meeting of the ASM contact Jim Sliwa, ASM Office of Communications at jsliwa@asmusa.org
EMBARGOED UNTIL: Monday, May 22, 9:00 a.m. EDT
(Session 041/Q, Paper Q-032)
Sara Firl
University of Minnesota
Minneapolis, MN, United States
Phone: 612 626 8865
firl0002@umn.edu
Our study determined that substantial numbers of antibiotic-resistant bacteria were present in municipal wastewater, and that the existing treatment infrastructure did not adequately prevent release of antibiotic-resistant bacteria into the environment. Many of the bacteria found in the wastewater treatment plant and in the plant effluent were tentatively identified as potential pathogens and were also resistant to multiple antibiotics, raising public health concerns. We believe that wastewater treatment plants could be modified to further prevent the release of resistant bacteria to the environment.

Sara Firl and Leslie Onan performed this study under the supervision of principal investigator Dr. Timothy LaPara at the University of Minnesota, Department of Civil Engineering. Funding was provided by the Center for Urban and Regional Affairs at the University of Minnesota and Geomatrix Consultants, Inc. The work is being presented as a poster at the 106th General Meeting of the American Society for Microbiology in Orlando on May 22.
The spread of antibiotic-resistant bacteria is a major public health concern. Infections previously treatable are increasingly resistant to antibiotics. Scientists believe that the spread of antibiotic resistance results from both misuse of antibiotics and transfer of resistance between bacteria. A potentially large reservoir for antibiotic-resistant bacteria is municipal wastewater. People release resistant bacteria with fecal matter into the wastewater stream, which is collected and treated at municipal treatment facilities before release to the environment. The objective of this study was to investigate how many resistant bacteria were present at municipal wastewater plants and if the existing infrastructure of waste treatment was adequate to remove resistant bacteria before discharge.

In our study, the effect of effluent treatment (clarification and disinfection) and biosolids treatment (sludge digestion) on the removal of antibiotic-resistant bacteria was investigated at three wastewater treatment facilities. We found substantial numbers of resistant bacteria at the wastewater treatment facilities and that, although effluent treatment reduced the numbers of bacteria, large quantities of resistant bacteria were discharged. Numerous bacteria isolated from the effluent stream were resistant to multiple antibiotics and closely related to potentially pathogenic bacteria. Our research suggests that the existing wastewater treatment infrastructure should be modified to better prevent release of these potentially dangerous bacteria to the environment.

+++++++ [Pathogen distribution in waste water sprinkler irrigation] [Article in German] ++++

Ahmed RE, Geuenich HH, Müller HE.
The spray irrigation with pretreated wastewater was investigated on the sewage farm of the Braunschweig Sewage Utilization Association. The emission of airborne bacteria was measured by means of Andersen sampler (AS), Reuter centrifugal sampler (RCS), and sedimentation plates (SP). There was a good correlation of results obtained by parallel measurings of AS and RCS. The RCS was more effective by the factor 11.5 than the AS sampling airborne microorganisms. However, the AS gave the distribution curve of differently big airborne particulates (Table 1). The medium decrease of bacteria from the sprinkler determined by AS and SP was similar (Fig. 1). With low wind velocity, an aerosol containing enterobacteria was yet detectable at a distance of 60-160 m down-wind from the spray sprinkler. The transport of bacteria as a function of the wind velocity is given in Fig. 2 increasing about 25 m for an increase of the wind velocity of 1 m/sec. During the spray irrigation, the composition of bacteria in the airborne particles is varying continuously. The following order of succession of die-away rates was found: Aeromonas, Plesiomonas, Vibrio greater than Acinetobacter, Pseudomonas greater than Enterobacter greater than Citrobacter greater than E. coli greater than Klebsiella greater than gram-positive bacteria (Table 3). Only seldom and under extreme conditions, gram-negative bacteria were detected in a range between 200-300 m beyond of concentrations as they were found also in controls without irrigation. This result substantiates a minimum protective distance of 300 m between sprinkler and human settlements. Zentralbl Bakteriol Mikrobiol Hyg [B]. 1984 May;179(2):151-61.

********************************************
********************************************

TO: SWRCB
Comments on the
Fm: Dr Edo McGowan

These comments are directed at the policy aspect as well as scientific data
The Anderson report of 2010, cited within this report as an authoritative document representing the thinking of the SWRCB was, in the past, reviewed and commented upon by me. Those comments were shared with the SWRCB. In those comments, I basically demonstrated that that the Anderson panel did not have the scientific knowledge and thus capacity to comment on antibiotic resistant contaminants. Notwithstanding having demonstrated such to the SWRCB, it choose to give a positive spin to the Anderson, et al document. I called them again on this. While the panel finally agreed with me, their report still maintained the spin that all was OK. This spin is now carried forward into the current document. That this new report carries the spin forward is a serious breach of integrity.

At least twice does the SWRCB seriously fictionalize reality in an attempt to down-play risks related to the use of recycled water: here at p.202 (see below) and previously via the Anderson report. This is not just an innocent happenstance in a series of inadvertent typos but now appears to be a rather calculated and deliberate attempt to subvert the truth.

These actions are typical of a clientele captured regulatory agency. Regulatory capture is a form of government failure that occurs when a regulatory agency, created to act in the public interest, instead advances the commercial or political concerns of special interest groups that dominate the industry or sector it is charged with regulating. The result of malfeasance in such cases may be seen in adverse impacts on public health and welfare as well as increased costs to the public and the health care systems.

In the current report at p. 202----------we see this statement:

"A Science Advisory Panel convened by the California State Water Resources Control Board (State Water Board) to address chemicals of emerging concern (CECs) in recycled water concluded that reuse practices did not cause or add to antibiotic resistance (Anderson et al., 2010)."

Interestingly, the Anderson panel following a review of its qualifications, admitted after being called on it, that it did not have the scientific qualifications to comment on this subject---antibiotic resistant organisms as found in recycled water. Thus, it is disingenuous at a minimum for the current report to indicate that that as an authority, Anderson, et al, "concluded that reuse practices did not cause or add to antibiotic resistance"

Nonetheless, even after being called on it, Anderson and the SWRCB continued to make assurances about recycled water. This, in spite of the fact that the SWRCB knew that the Anderson panel was not qualified to make such statements and even after admitting that it had no technical and scientific capacity to do so. Again, this smacks of clientele capture.

Here below is what the Anderson report actually says and that statement is at odds with the statement on page 202 of the current report. Thus, the statement found above (page 202) are more than misleading--------they are ignoring the reality of recycled water to carry serious pathogens, including those that are multi drug resistant as well as their genes, a serious breach of public trust. The SWRCB has a duty to warn, not cover up. It has evidently chosen the latter course. I have previously commented on this issue and thus find it necessary to incorporate by reference herein all previously submitted comments to the SWRCB.
"The Panel also chose not to consider the occurrence of waterborne microbial pathogens or their acquisition of antibiotic resistance. Given the multiple barrier concept and water treatment process redundancy requirements in place, the Panel believes that the potential public health risk associated with exposure to pathogens in recycled water used for landscape irrigation or groundwater recharge is very small. However, the Panel acknowledges that some uncertainties exist regarding the occurrence of emerging waterborne microbial pathogens and encourages additional research into their fate in water reuse systems. The Panel did provide a cursory review of antibiotic resistance in relation to water reuse practices (see Appendix C) and realized that the issue was complex and that a thorough treatment required more resources than the Panel had access to." End of quote from Anderson.

Several of the toxins found in wastewater are also mitochondrial poisons and thus can and do cause shifts in mitochondrial genes. These mutations can be duplicated with mitochondria fission and then when refusion occurs, can be established, and thus set up a litany of pathologies. Many of these pathologies will not be seen until later life. Such pathologies, if found in the female will be passed genetically to offspring. The document discusses none of this. This is a big flaw. This also impacts any type of risk assessment. The current report discusses risk assessments:

**Antibiotic Resistance**

"Until risk assessment tools are established, evaluating the quantity of ARG and/or ARB through water treatment processes offers the best approach for limiting any potential risk related to antibiotic resistance. For instance, antibiotic resistance detection methods can be used to identify treatment practices that efficiently remove antibiotic resistance determinants or as part of a framework verifying the operational effectiveness of particular treatments.

Regulatory levels related to microbial constituents have been established for the protection of public health, but not for antibiotic resistance. Currently, there are no regulations pertaining to antibiotic resistance in wastewater or potable water due to several factors, including the lack of data on the dose-response relationship between antibiotic resistance determinants in water and adverse human health effects (Ashbolt et al., 2013); however, information on the occurrence and concentration of some antibiotic resistance elements in water and wastewater is available. Until risk assessment..."
tools are established, evaluating the quantity of ARG and/or ARB through water treatment processes offers the best approach for limiting any potential risk related to antibiotic resistance. For instance, antibiotic resistance detection methods can be used to identify treatment practices that efficiently remove antibiotic resistance determinants or as part of a framework verifying the operational effectiveness of particular treatments."

Until risk assessment tools are established, evaluating the quantity of ARG and/or ARB through water treatment processes offers the best approach for limiting any potential risk related to antibiotic resistance. For instance, antibiotic resistance detection methods can be used to identify treatment practices that efficiently remove antibiotic resistance determinants or as part of a framework verifying the operational effectiveness of particular treatments.

What do they mean by this? Who is going to test for these organisms or their genes? Where is their plan to actualize this? Funding requests? Organization?

Sent Items

Sensitivity of antibiotic resistant and antibiotic susceptible Escherichia coli, Enterococcus and Staphylococcus strains against ozone.
Heß S, Gallert C.
PMID: 26608763

Resistance behaviour of inducible clindamycin-resistant staphylococci from clinical samples and aquatic environments.
Heß S, Gallert C.
PMID: 25106860

Demonstration of staphylococci with inducible macrolide-lincosamide-streptogramin B (MLSB ) resistance in sewage and river water and of the capacity of anhydroerythromycin to induce MLSB.
Heß S, Gallert C.
PMID: 24308503

Detection of the macrolide-lincosamide-streptogramin B resistance gene erm(44) and a novel erm(44) variant in staphylococci from aquatic environments.
PMID: 26207047
Similar articles
Select item 254791875.
Removal of micropollutants, facultative pathogenic and antibiotic resistant bacteria in a full-scale retention soil filter receiving combined sewer overflow.
PMID: 25479187

Similar articles
Select item 272209726.
Growth Behavior of E. coli, Enterococcus and Staphylococcus Species in the Presence and Absence of Sub-inhibitory Antibiotic Concentrations: Consequences for Interpretation of Culture-Based Data.
Heß S, Gallert C.
PMID: 27220972

Similar articles
Select item 259645197.
Staphylococcus argensis sp. nov., a novel staphylococcal species isolated from an aquatic environment.
Heß S, Gallert C.
PMID: 25964519

Similar articles
Select item 254971748.
Removal of total and antibiotic resistant bacteria in advanced wastewater treatment by ozonation in combination with different filtering techniques.
PMID: 25497174

************************

SBDDW-16-02 Surface Water Augmentation February 14, 2017
waterboards.ca.gov

Note that these are raw water levels. The Fahrenfeld paper was a look at tertiary treated recycled water. The state uses counts per liter and Fahrenfeld used counts per ml. To bring the Fahrenfeld up to a parallel with the state, one would need to go up 3-magnitudes, that is roughly 10-to the 7 through the 9th. Using state data and running the process backward to get original numbers in raw sewage might prove interesting--I don't have that kind of time. My guess is that the numbers for the ARGs would dwarf those of the pathogens the state is using.

The running of ARGs could be offered as a backup and Amy or one of her grad students would be a source
for running this past.

These raw wastewater levels are considered the highest organism densities that are expected in raw municipal sewage. The maximum raw sewage pathogen density was used, rather than the 95th percentile density (or some other percentile), to provide further confidence that the public would be protected during worst-case wastewater pathogen occurrences. Table 1 summarizes the outcome of the overall approach used.

Table 1
Enteric virus Giardia cysts Cryptosporidium oocysts
Raw sewage maximum density $1 \times 10^5$ virus/L.

Edo McGowan
The SCHEER analysis of the proposed JRC minimum quality requirements relating to use of recycled. The document discusses of mentions antibiotic resistance on pp. 6, 13, 15, 16, 17, 18, 22, 23.

The term "pathogen is seen on pp. 13, 17, 21, 22

It is SCHEER's opinion the JRC (Joint Research Centre) inadequately addressed CECs and antibiotic resistance, including genes.

With respect to using recycled water for irrigating crops, interestingly, SCHEER notes issues with the JRC's analysis that parallel concerns similarly ignored by the regulatory community here is the U.S. and California, specifically, I would point to the SWRCB. This then, would tend to raise some
serious questions around the extent and level of regulatory clientele capture by the industry. These concerns voiced by SCHEER relate to antibiotic resistance, the genes of such organisms and consequently the potential for contamination of the environment as well as the watered crops. Thus, how does this affect transfer to the human gut microbiota, setting up reservoirs of resistance within an ever increasingly complex population of pathogens in an economic era of disinterest amongst the pharmaceutical industry.

SCHEER suggests the precautionary principle. Bradford Hill discusses causation, but cautions that not all the factors are necessary and that the underlying issue is one of taking a precautionary approach.

With respect to dose-response, the fact that reservoirs of resistance can be established either environmentally or internally, the issue of gene transfer throws a complex confounder at the issue. Thus, awaiting the development of "risk assessment" or "dose-response" is just stalling the correction and admission that sewer plants are principal generators of resistant organisms. This has been discussed extensively in the literature, dating back as the mid 1950s. Certainly a proactive regulator would have more to show for the time. A clientele captured regulator would not show much progress, as amply demonstrated by the SWRCB.

******************************